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THE EFFECT OF INOCULATION, FERTILIZER TREATMENT AND CERTAIN MINERALS ON THE YIELD, COMPOSITION AND NODULE FORMATION OF SOYBEANS[1]

C. R. FELLERS

Formerly Research Fellow, Rutgers College

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THE EFFECT OF INOCULATION ON THE YIELD AND COMPOSITION OF SOYBEANS

It has been shown many times that inoculation increases both the yield and the protein content of soybeans, but little or no data have been presented to show the effect upon the oil content.

Lipman and Blair (28) showed that in well inoculated soybean plants the per cent of nitrogen was greater than in uninoculated or in poorly inoculated plants; and that the yield also was higher. The West Virginia (5), Michigan (47), Kansas (6), and Wisconsin (9) experiment stations also have shown that a good inoculation increases the yield as well as the protein content of the plant. Some investigators have reported slight, or no, increase in yield due to inoculation. Among these are the Nebraska (23) and Wisconsin stations. The Illinois (51) station showed that inoculated cowpeas were much richer in nitrogen than uninoculated cowpeas. Not only is it a well established fact that inoculation increases the yield and protein content of legumes in general, but as Lipman (27) and Lyon and Bizzell (34) have shown, the association of non-legumes with legumes in mixed cultures results in an increased percentage of protein for the former. That this fact was well known and put into actual practice for centuries past, was shown by Dr. Lipman.

Although much work has been done on the effect of inoculation on the protein content of legumes and of soybeans in particular, but little has been done on the factors causing variations in the oil content of the seeds.

Perhaps the most work done on the effect of inoculation upon the composition of the soybean plant was done by Lipman and his associates (28, 29), Fred (9), and Smith and Robinson (45). These workers show that a substantial increase in protein results from inoculating soybeans. The former two writers also showed that liming increased the percentage of protein in soybeans.

[1] The writer wishes to take this occasion to thank Dr. J. G. Lipman, Prof. A. W. Blair and Prof. Frank App, of the faculty of Rutgers College, for advice and suggestions during the progress of this work.

Mention has been made a few times that the oil content of soybeans decreases as the protein content increases. Among the authors who have mentioned this phenomenon are Robert (38) and Allison (3). They claim that high oil and high protein content do not go together in soybeans.

Grantham (14) in 1912 states:

> Very high oil and very high protein content in soybeans do not seem to be closely correlated. The variety yielding the most protein was lowest in oil. Whether the high per cent of protein and oil can be increased by selection and breeding remains to be seen, as little or no work has been done along this line.

The considerable variation in composition among varieties offers an opportunity for developing strains of soybeans for specific purposes.

Robert (39) concludes from the results of his analyses of soybeans that high oil and protein content are not correlated, but that high protein yielding varieties have a low oil content. Most of the investigators just quoted are cognizant of this fact, but little or no work has been done on the effect of inoculation upon the oil content of soybeans.

The author studied this problem in both greenhouse pot experiments and in field plots. The variety of soybean used was the "Black Eyebrow," a very promising variety for New Jersey. Morse (36), of the United States Bureau of Plant Industry, characterizes it as follows:

> The seeds are black and yellow. It is an early variety obtained from Manchuria and maturing slightly earlier than Ito San, excelling the latter in both hay and seed production. It is most suitable as a grain variety in the northern states.

Jenkins (20) of Connecticut states: "Black Eyebrow soybeans yielded 21.6 bushels per acre of seed and matured in 109 days. Only one variety matured earlier, and that one by only two days." Lipman and Blair (29), of the New Jersey station, state that it requires about 120 days for maturity. The 1915 crops yielded only 10.8 bushels of seed per acre with a protein content of 40.5 per cent.

Methods used for the analysis of soybeans

The samples were partly dried to facilitate grinding and then ground in a small power mill to a flour. All samples were ground to pass a 40-mesh sieve as it was found that the oil was incompletely extracted from several samples which were more coarsely ground. Good duplicate determinations were obtained by using material for analysis which had passed a 40-mesh sieve. The Soxhlet method of extraction was used for the determination of the oil. Electric light bulbs were used to furnish the necessary heat. Extractions were run 8 hours, as it was found that practically all of the oil could be extracted in that length of time. The flasks containing the oil were dried to constant weight in an electric oven at a temperature of 100°C. It

was noticed that if the flasks remained in the oven too long an increase in weight took place. This was evidently due to the absorption of oxygen by the oil, as it became thicker and tougher the longer it remained in the oven. From 30 to 50 minutes' drying was found to give satisfactory results.

The nitrogen content of soybeans was determined by the Gunning modification of the Kjeldahl method; namely, by using for a half-gram sample of bean flour, 25 cc. of concentrated sulfuric acid and 5 gm. of potassium sulfate. A modification was further made by adding to each flask about 1 to 2 gm. of copper sulfate in addition to the above chemicals. It was found to facilitate digestion greatly. The function of the copper sulfate is probably that of a catalyzer, although it may assist in the oxidation of the organic matter by taking part in the reaction. A complete digestion may be carried out in this way in from 20 to 30 minutes.

Pot experiments

The pots used were 10-pound glazed earthenware pots. The soil was a virgin Sassafras sandy loam containing no nitrogen-fixing organisms of the soybean variety. The fertilizer treatment was 2 gm. of acid phosphate, 2 gm. of muriate of potash, and 5 gm. of ground limestone per pot. The seeds, after being sterilized with $HgCl_2$ (1:1000) and thoroughly washed for some hours in distilled water, were inoculated with soil in varying quantities and commercial cultures, and planted at the rate of 8 to the pot. Pots 19 to 22 were inoculated with a pure culture of *Bacillus radicicola*, isolated a few days before from a soybean nodule, and grown since that time on Ashby's nitrogen-free medium.

These data show that there is a considerable variation, with the different methods of inoculation, in the yield of total dry matter, the number of nodules per plant, and the per cent of oil and protein in the seeds of the plants. The four check pots agreed very well in yield of total dry matter, at about 9 gm. per pot. Pots 11 and 12 inoculated with Mulford's Nitrogerm also agreed with the checks, showing that little or no inoculation had been produced. Pots 3 to 10 form an interesting series. It appears from the data obtained that the yield of total dry matter, number of nodules and per cent of protein vary directly as the amount of soil added up to 5 gm., when the maximum is reached. Ten grams of inoculated soil per pot did not give increases over 5 gm. The other cultures rank about the same as regards nodule production, yield and per cent of protein. Apparently, the most efficient culture of all was the freshly isolated culture of *B. radicicola* from soybean nodules. In the four pots where used it gave increased yield and nodule production, and high protein and low oil content. The inoculating efficiency of two soils both containing the soybean organism, appears to vary, since almost twice as many nodules were produced in one case as in the other. The per cent of oil and protein is proportionally large or small ac-

cording as the inoculation was good or poor. The oil varies from 17.7 to 22.5 per cent, the greatest amount being present in the checks, and the lowest in those pots having the most thorough inoculation, and consequently the greatest number of nodules per plant.

TABLE 1

Effect of inoculation upon the yield and composition of soybeans

POT	NATURE OF INOCULATION	TOTAL DRY MATTER	AVERAGE TOTAL DRY MATTER	AVERAGE NUMBER NODULES PER PLANT	PER CENT PROTEIN IN SEED	PER CENT OIL IN SEED
		gm.	*gm.*			
1	Check: no inoculation	8.5	8.90	0	38.6	21.9
2		9.3				
3	1 gm. soil from soybean field	12.7	13.15	2.3	40.0	20.7
4		13.6				
5	2 gm. soil from soybean field	14.9	14.80	5.2	41.2*	19.1*
6		14.0				
7	5 gm. soil from soybean field	17.7	17.25	24.0	41.9	18.0
8		16.8				
9	10 gm. soil from soybean field	17.6	17.10	31.0	41.9	17.7
10		16.6				
11	Mulford's Nitrogerm	9.2	8.15	0.4	39.0	22.4
12		7.1				
13	Earp-Thomas Farmogerm	18.1	18.10	25.0	41.9	19.0
14		18.1				
15	Standard Nitrate Agencies' culture	17.3	17.75	18.0	42.0	18.1
16		18.2				
17	U. S. Dept. Agr. Culture	18.6	18.25	21.0	41.6	18.4
18		17.9				
19	Freshly-isolated pure culture: 1 cc.	20.1	19.90	24.1	40.2	17.9
20		19.7				
21	Freshly-isolated pure culture: 10 cc.	20.1	20.50	26.0	41.7	17.9
22		20.9				
23	2 gm. soil from soybean field no. 2	17.2	16.80	9.1	41.6	19.6
24		16.4				
25	Check: no inoculation	9.0	9.15	0.8	38.2	22.5
26		9.3				

*Pot 5 only; pot 6 was lost.

From this experiment it could be concluded that in a rather poor sandy soil inoculation decreased the oil content of soybeans in proportion to the thoroughness of the infection. In the same way the protein content is increased.

Field experiments to show effect of inoculation upon yield and composition of soybeans

Since such interesting data were obtained from pot experiments it was decided to continue the experiment in the field. The soil was an acid Sassafras sandy loam, which had never grown soybeans and which was shown to contain no *B. radicicola* (variety soybean) by culture of soybeans on the land. The plots were 5 by 10 feet, or 0.001141 acre in size. The vegetation existing on the land when broken was sparse, consisting principally of wild celery, crab-grass and Canada blue-grass. The variety of soybeans used was the Black Eyebrow. Plots were planted in duplicate in all cases with an intervening space of 12 inches. Exactly the same fertilizer treatment was given each duplicate plot. This consisted of applications at the acre rate of 400 pounds of acid phosphate, 200 pounds of muriate of potash and 2000 pounds of ground oyster shells in excess of the lime requirement as determined by the Veitch method. The soil used for inoculating some of the plots had grown a luxuriant crop of soybeans the year before, the plants being well supplied with nodules. This soil was spread over the plots evenly and worked in with a rake. The commercial culture used was Mulford's Nitrogerm. This was diluted according to directions and the beans allowed to soak 2 hours in a dark room. They were planted while still moist and covered immediately. The first nodules were noticed after 12 days on plot 72A. From the beginning of the experiment the inoculated plots were evident from their dark color and increased leafiness. The seeds had fully matured in 95 days, and after drying, weighing, threshing and cleaning, the beans were analyzed for oil and protein content. The roots, tops and straw also were weighed and analyzed, but the results are given in another paper. Counts of nodules on the roots also were made.

The data in table 2 show that the yield of soybeans is very materially increased by inoculation, and that this increase becomes larger with the thoroughness of the infection. The duplicates in some instances do not agree very well, but the large number of check plots without any inoculation show that any increases in crop yields are undoubtedly due to the presence of the nitrogen-gathering bacteria. Not only total dry matter was increased, but also the seed and straw yields showed substantial increases over the checks.

As in the pot experiments, Mulford's Nitrogerm did not inoculate the soybeans. The number of nodules per plant and yield of total dry matter and seed increased with the amount of soil used for inoculation. The field experiment substantiated the indications procured in the pot experiments.

Effect of inoculation upon the composition of soybeans

The seed from these plots was carefully sampled, dried, ground and analyzed for oil and protein content. The data obtained are given in table 3. Only plot numbers are given, as reference can easily be made to table 2 which shows the treatments of the several plots.

TABLE 2

Effect of inoculation upon the yield of soybeans

PLOT NO.	INOCULATING MATERIAL	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	TOTAL DRY MATTER INCREASE OVER CHECKS	SEED INCREASE OVER CHECKS	NUMBER NODULES PER PLANT*
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	
70	Check: no inoculation	3.11	1.16	1.95	2.85	1.06			0.1
70A		2.59	0.95	1.64					
71	Soil: 11 lb.	3.83	1.68	2.15	3.33	1.50	0.54	0.48	5.0
71A		2.83	1.31	1.52					
72	Soil: 5 lbs.	3.82	1.78	2.04	3.53	1.75	0.79	0.08	10.2
72A		3.24	1.71	1.53					
73	10 cc. Mulford Nitro-	2.30	0.85	1.52	2.24	0.86	−0.45	−0.05	0
73A	germ	2.11	0.90	1.21					
74	Check: no inoculation	3.06	1.02	2.04	2.62	0.89			0
74A		2.17	0.76	1.41					
75	30 cc. Mulford Nitro-	2.72	1.07	1.65	2.65	0.98	−0.26	−0.04	0.3
75A	germ	2.57	0.89	1.68					
76	10 cc. Nitrogerm − 1	3.92	1.90	2.02	3.98	1.84	1.26	0.83	7.2
76A	lb. soil	4.05	1.78	2.27					
77	Check: no inoculation	2.95	1.13	1.82	3.00	1.10			0.3
77A		3.05	1.20	1.85					
78	Soil: 3 lbs.	3.67	1.76	1.91	3.75	1.64	1.25	0.48	7.0
78A		3.83	1.51	2.32					

* Nodule counts were taken when plants were 32 days old.

Table 3 shows that the inoculation of soybeans increases the per cent of protein and decreases the per cent of oil in the seeds. The average increase in protein content for the eight inoculated plots is 7.04 per cent and the average decrease in oil content due to inoculation in these same eight plots is 3.18 per cent.

In connection with the oil determinations, crude drying tests were made from all of the samples to see if the quality of the oil varied, but as far as

was shown by the simple drying tests made, there is little if any difference in the oil from these beans. The method of procedure was to warm the flasks containing the oil residue to decrease its viscosity, then to make smears of this oil on pine boards by means of small brushes. These smears were examined several times daily. Because of the lack of uniformity in procedure, and in the treatment of the oil samples (some had to be dried longer than others to become constant in weight), too much emphasis cannot be placed on these

TABLE 3

Effect of inoculation upon the composition of soybeans

PLOT	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECKS	INCREASE IN PROTEIN OVER CHECKS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
70	19.1	36.0	19.20	36.10		
70A	19.3	36.2				
71	16.8	39.4	16.90	39.95	−3.77	5.65
71A	17.0	40.5				
72	18.1	44.6	18.55	43.80	−3.22	9.50
72A	19.0	43.0				
73	20.9	36.8	21.10	34.70	+0.33	0.40
73A	21.3	32.6				
74	21.2	33.3	21.95	33.30		
74A	22.7	33.3				
75	22.0	33.3	21.80	33.45	+1.03	0.05
75A	21.6	33.6				
76	17.3	38.5	17.65	38.75	−3.12	4.45
76A	18.0	39.0				
77	20.7	33.3	21.15	33.85		
77A	21.6	34.4				
78	18.3	42.7	18.05	42.85	−2.62	8.55
78A	17.8	43.0				

results. Some samples were darker colored than others, but as a rule the differences were slight.

Effect of liming the soil upon the yield and composition of soybeans

Extensive researches have been carried on in this field of work. The most complete are those of Lipman and Blair (29, 30, 32) who have made a number of tests, and in every case found the yield and protein content increased

as a result of liming. They made use of both vegetative greenhouse experiments and field plot tests in their work. On an average for three years, the increase in protein content due to liming was 3.06 per cent. The increase in yield varied with the several varieties from 0 to 11 bushels; with an average of about 5 bushels per acre for a 3-year period. Liming increased nodule production 42 per cent. It was estimated that a crop of soybeans will fix about 65 pounds of nitrogen per acre per year. In another article, Lipman and his coworkers (33) showed that an application of 1000 pounds of lime per acre was as beneficial as 4000 pounds for soybeans.

Fred and Graul (9) working with an acid Colby silt loam, as a result of their investigation state:

> Inoculation is very beneficial to soybeans, but liming alone did not cause any very consistent gain in total nitrogen; although where both lime and inoculation were used there was a slight gain in yield and total nitrogen, but hardly enough to warrant recommending lime for soybeans.

They found small applications of calcium carbonate to be as beneficial as larger applications for the common legumes when grown on acid soils. Large applications were injurious.

Duggar and Funchess (71) found that liming increased the yield of soybeans 49 per cent. Hopkins (16), Frear (8) and Lipman (33) have reported results showing small quantities of lime to be as beneficial as larger amounts in the case of various legumes.

Prianischnikov (38) found small dressings of calcium carbonate beneficial to lupines, but large applications were injurious. Ulbricht (48) and Khandurin (22) also showed the same point with other legumes. Kassovich and Althausen (24) working on the acid podzol soils of Russia, found that plants highly sensitive to acidity, as mustard and clover, were helped the most by additions of lime sufficient to neutralize the soil acidity, but were most injured by excessive amounts of lime.

Schulze (41) found 1 per cent of calcium carbonate in soils very injurious to lupines and serradella, and 5 per cent was sufficient to prevent growth. Even 0.5 per cent was decidedly injurious.

Kopeloff (25) has recently shown that the effectiveness of ground limestone is partly measured by its fineness of division, hence one ton of 200-mesh material may act in the same manner as three or four tons of coarsely ground limestone. Abbott (1) reports an increase in yield of soybeans due to the use of lime of 7 bushels per acre.

This literature concerning the use of lime for soybeans could easily be augmented, but enough has been cited to show that liming increases the yield of most legumes, that small amounts are nearly as good as large amounts, and that, in general, liming increases the yield and protein content of the seed.

Pot experiments to show the influence of lime upon the yield and composition of soybean seeds

Glazed, round, earthenware pots holding 10 pounds of soil were used in this experiment. The soil was acid Sassafras sandy loam. The lime requirement according to the Veitch method was 6000 pounds of CaO per acre. The ground limestone used was 40-mesh material, although a portion (about 10 per cent) did not pass a 40-mesh sieve. Seven seeds of the Black Eyebrow variety were allowed to mature in each pot. The moisture was kept at optimum as nearly as possible. Two grams each of acid phosphate and muriate of potash were added to each pot, thus making the limestone the only variable factor. Inoculation of the seed was made from a suspension of crushed soybean nodules. Table 4 gives the data obtained in this experiment.

Table 4 shows that both ground limestone and burnt lime increase the yield of total dry matter of soybeans on an acid soil. In some cases the crop yield is nearly doubled, and in every case, except where there was a tremendous excess of $CaCO_3$ in the soil, there was a material increase. Ground limestone and burnt lime seem to be about equally efficient in increasing crop growth and in nodule production. It is seen at a glance that small quantities of lime or ground limestone are practically as efficient as larger amounts in stimulating plant growth and nodule production, and in increasing the protein content. The oil content of the seeds varies considerably. This is to be expected since in some cases only a few grams of seeds were obtained from a pot and these might well vary in composition. The reason analyses were not determined on the separate pots was that the yield of seed was not great enough from a single pot to insure an accurate analysis, hence the seeds from duplicate pots were mixed and then ground and analyzed. The counts of nodules are not absolutely accurate, as by the time the plants had matured some of the nodules had begun to decay, making the counting difficult at times. As in the case of former experiments, the oil content of soybean seeds decreases as the protein increases, and vice versa. The highest percentage of oil was obtained from the check pots to which no lime was applied. Ordinarily, the oil content would probably not be much affected by liming in addition to inoculation, but on the very acid soil which was used in this experiment the nitrogen-gathering bacteria were not very efficient, as is shown by the lower number of nodules on the roots of the check plots. From this experiment it may be concluded that liming increased the total dry matter, the number of nodules per plant, and the protein content of the seed of soybeans grown in an acid soil. Little or no differences are evident whether burnt lime or ground limestone is used to correct the soil acidity. Large quantities of either of these materials had an inimical effect on plant growth, Soybeans do not seem to require much lime in the soil for their best growth, as when only sufficient burnt lime or ground limestone was added to the soil

TABLE 4

Effect of lime and ground limestone upon yield and composition of soybeans

POT NO.	TREATMENT: 2 GM. A. P. AND KCl	TOTAL DRY MATTER	AVERAGE TOTAL DRY MATTER	NUMBER OF NODULES	PROTEIN IN SEEDS	OIL IN SEEDS
		gm.	*gm.*		*per cent*	*per cent*
1	Checks: no limestone	10.7	10.90	18.3	36.0	22.1
2		11.1				
3	Acidity half neutralized	17.3	17.15	31.1	39.7	18.5
4		17.0				
5	Acidity neutralized	17.6	17.85	27.2	42.6	18.2
6		18.1				
7	1000 pounds excess limestone	19.4	18.85	26.1	44.0	18.5
8		18.3				
9	2000 pounds excess limestone	19.2	18.40	31.7	43.6	19.6
10		17.6				
11	5000 pounds excess limestone	14.7	15.85	24.3	45.3	18.2
12		17.0				
13	10,000 pounds excess lime-	13.3	12.15	25.9	45.8	17.3
14	stone	11.0				
15	Check: no limestone	11.7	11.00	13.7	37.2	21.6
16		10.3				
17	Acidity half neutralized with	18.7	19.05	33.6	41.7	19.0
18	CaO	19.4				
19	Acidity neutralized with CaO	18.0	17.55	31.4	43.0	18.7
20		17.1				
21	1000 pounds excess CaO	18.3	18.75	28.3	43.4	18.0
22		19.2				
23	2000 pounds excess CaO	15.7	14.80	28.3	43.9	18.6
24		13.9				
25	5000 pounds excess CaO	13.3	13.30	21.2	44.6	17.6
26		13.3				
27	Check: No CaO	10.6	10.85	15.4	36.9	21.7
28		11.1				

to neutralize one-half of the acidity present (Veitch method), maximum crop yields were obtained.

Liming increased the per cent of protein and decreased the per cent of oil in soybeans grown on an acid soil. This is not a weighty argument against liming, because the greater amount of oil would be produced in a full crop of soybeans rather than in half a crop or two-thirds of a crop, which would be obtained on unlimed soil, in spite of the latter's greater oil content. In other words, the greater oil percentage in unlimed soybeans or in uninoculated plants does not overbalance the increased yield due to liming and inoculation.

As in the case with the inoculation experiments, the vegetation results were checked up in field plots the following summer. Plots 5 by 10 feet, as previously described in this paper, were used. The soil was an acid Sassafras sandy loam, of rather poor crop-producing power. Only enough plots were available to test the effect of ground limestone in the field, hence the burnt lime was not used. The similarity of results of the vegetation experiments, however, led the author to believe that the results would have closely simulated those obtained for ground limestone. All of the plots were well inoculated except plot 79, which was neither limed nor inoculated, and plot 80. The form of lime used in this experiment was ground oyster shells, the greater part of which passed a 10-mesh sieve. All of the plots received a fertilizer dressing equivalent to 400 pounds of acid phosphate and 200 pounds of muriate of potash per acre. The inoculation was performed by adding 3 pounds of well infected soil to each plot, and working in with a rake. The beans were planted June 17, 1916, the variety used being the Black Eyebrow. The beans were kept well cultivated during the summer, and were harvested September 21, 1916. Thus 96 days were required for maturity. The limed plots matured about 6 to 8 days later than the unlimed check plots. The plants of the former were also much larger and leafier.

The data are given in tables 5 and 6.

Upon examining tables 5 and 6 it is seen that the field experiments closely check up the vegetation experiments with limestone, already discussed. As in the latter, the field experiments show that liming the soil is of great benefit in increasing the yield of total dry matter (tops) and seeds. The application of enough lime to neutralize one-half of the soil acidity gave an increased crop yield of 30 per cent; where the acidity was exactly neutralized 37 per cent; and where there were 6000 pounds of ground oyster shells in excess of the lime requirement, an increase of 42 per cent was noted. From these figures it is at once seen that it will not pay to add large quantities of lime to soils even though they are very acid, if soybeans are to be grown. Although the crop yields continue to increase when lime is applied in excess of the amount required to neutralize the soil acidity, yet these increases are small, and when we consider the cost of lime, application, and the amount leached away in the drainage water each year, it is not a paying investment to add large quan-

tities to a field. It is far better to make small applications of, say, 1000 to 3000 pounds of ground limestone every two or three years.

An interesting point is brought out in the results obtained from plots 79, 80 and 81. They also show the relative importance of inoculation and liming

TABLE 5

Effect of varying quantities of ground oyster shells upon the yield and composition of soybeans

PLOT NO.	TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs*	*lbs.*	*lbs.*
79	Check (I): No inoculation, no lime	1.92	0.80	1.10	1.96	0.76	−0.44*	−0.33	0.†
79A		2.03	0.73	1.30					
80	Check (II): Lime, no inoculation	2.95	1.13	1.82	3.00	1.16	+0.60	0.06	0.3
80A		3.05	1.20	1.85					
81	Check (III): No lime, inoculation	2.30	1.14	1.16	2.30	1.05			1.5
81A		2.30	.96	1.34					
82	Acidity half neutralized	3.98	1.97	2.01	3.58	1.82	1.18	0.73	7.4
82A		3.17	1.67	1.50					
83	Acidity just neutralized	4.04	1.88	2.16	3.87	1.76	1.42	0.64	10.0
83A		3.70	1.63	2.07					
84	2000 lbs. excess $CaCO_3$	3.67	1.76	1.91	3.75	1.64	1.25	0.48	7.0
84A		3.83	1.51	1.32					
85	4000 lbs. excess $CaCO_3$	3.99	1.88	2.11	3.64	1.76	1.09	0.65	7.5
85A		3.29	1.64	1.65					
86	Check: No $CaCO_3$	2.65	1.26	1.39	2.65	1.23			1.4
86A		2.65	1.19	1.46					
87	6000 lbs. excess $CaCO_3$	4.46	2.06	2.40	4.25	1.86	1.57	0.62	12.4
87A		4.03	1.66	2.37					
88	10,000 lbs. excess $CaCO_3$	4.52	2.20	2.30	4.28	1.93	1.38	0.81	15.3
88A		3.66	1.92	1.74					
89	Check: No $CaCO_3$	2.87	1.35	1.52	2.75	1.28			1.9
89A		2.63	1.21	1.42					

* Check plot III (81–81A, 86–86A, and 89–89A), are used as the basis of calculation.

† Nodule counts were taken when the plants were 33 days old.

for soybeans. From the data it appears that inoculation is the less important, as plot 80, which has lime but no inoculation, gave an increase in crop yield of 30 per cent over plot 81 which had no lime but which was in-

oculated. However, too much emphasis should not be placed on this single experiment, as soil conditions and different varieties of soybeans might give other results. That liming alone, or inoculation alone, gives an increase over the no-lime, no-inoculation check, is brought out in the tables. It is

TABLE 6

Effect of limestone upon the composition of soybean seed

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE OF OIL OVER CHECKS*	INCREASE OF PROTEIN OVER CHECKS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
79	21.8	33.0	21.95	32.80	1.03	−1.27
79A	22.1	32.6				
80	20.7	33.3	21.15	33.85	0.23	−0.22
80A	21.6	34.4				
81	21.2	33.6	21.40	34.05		
81A	21.6	34.5				
82	17.5	39.8	18.10	38.72	−2.82	4.65
82A	18.7	37.6				
83	18.2	43.9	18.10	44.00	−2.82	9.93
83A	18.0	44.1				
84	18.3	43.0	18.30	42.85	−2.62	8.78
84A	18.3	42.7				
85	18.1	44.3	18.05	44.05	−2.87	9.98
85A	18.0	43.8				
86	20.5	34.6	20.10	34.30		
86A	19.7	34.0				
87	17.6	43.8	17.50	44.10	−3.42	9.98
87A	17.4	44.4				
88	18.1	45.2	17.85	45.70	−3.07	11.63
88A	17.6	46.2				
89	21.0	33.5	21.25	33.85		
89A	21.5	34.2				

* The inoculated, no-lime plots, are used as checks, the same as in table 5.

unlikely that it would pay either to inoculate or to lime the soil alone. The crop yield is so dependent upon both of these factors that neither can be well omitted in the successful growing of soybeans on sour soils.

In general, seed and straw yields follow the same rule as total dry matter, since they, taken together, make up the latter. Bacterial infection in highly

acid soils does not readily take place, as is shown by the small number of nodules on the check plots receiving no lime. All of the plots except 79 to 80A were thoroughly inoculated with well infected soil. Slight infection of plots 80 and 80A was probably due to the transference by wind or animals of a little of the infected soil from plots 81 and 81A. The maximum nodule production took place where there was the greatest amount of lime, namely, in plots 88 and 88A. There is but little difference, however, in the number of nodules per plant on plots receiving small or large applications of lime.

The oil content of soybeans decreases in direct proportion as the application of lime increases in amount; conversely, the protein increases. The oil content of the soybean seeds varied from 17.5 per cent in plot 86 to 21.95 per cent in plot 79. The latter plot had neither lime nor inoculation. The no-lime plots gave an average percentage of oil of 20.92. Taking the results by and large, it is safe to say that liming the soil in this experiment decreased the oil content of the beans about 2.8 per cent. Large applications caused a decrease of over 3 per cent. Plot 80, which was not inoculated but limed, gave practically the same per cent of oil as plot 81, which received no lime but was inoculated. Plot 79, having neither lime nor inoculation, had 1 per cent more oil than these plots. As was found to be the case with crop yield, small amounts of lime were nearly as beneficial as large amounts in increasing the protein content of the soybean seed. The lowest amount of this constituent was found in the check plots where the per cent of oil was highest. Lime without inoculation, or vice versa, is not very efficient in increasing the amount of protein in soybean seeds. Both are necessary.

THE YIELD AND COMPOSITION OF SOYBEAN SEEDS AS AFFECTED BY CERTAIN FERTILIZERS AND CHEMICAL SALTS

A hasty review of the literature on this subject reveals the fact that a considerable amount of data has been accumulated on the action of various fertilizers and salts on the protein content of soybeans, but little regarding their action on the oil content.

That fertilizers, especially in the form of phosphorus-containing materials and potash, are quite essential to the production of a maximum crop of soybeans, is the consensus of opinion of the experiment stations of the United States. Soybeans, like most other legumes, draw heavily upon the stores of mineral plant-food in the soil, hence some provision must be made to repay the soil for what it has given to the crop, especially if the fodder or beans are not fed on the farm.

The following experiment stations have recommended applications of fertilizer as follows:

New Jersey (50): 250 pounds acid phosphate; 50 pounds KCl (on light soils).

Rhode Island (2): Applications of acid phosphate, muriate of potash and lime.

Connecticut (21): 200 to 300 pounds of acid phosphate and lime on poor soils.

West Virginia (5): Liberal application of acid phosphate and lime.

Delaware (14): Crimson clover sod supplemented by 250 to 350 pounds of a mixture of 400 pounds of acid phosphate and 100 pounds of muriate of potash.

Ohio (53): Fertilizers not needed on good soils; on poor soils stable manure or complete fertilizers are used with profit.

New York (15): Applications of phosphoric acid and potash if these are deficient in the soil; a small application of nitrate of soda is helpful in giving a vigorous start to the plants.

North Carolina (52): 200 to 400 pounds of acid phosphate and 25 to 50 pounds of muriate of potash. Acid phosphate alone is very beneficial.

Tennessee (35): 300 pounds of acid phosphate, and 250 pounds of wood ashes or 25 pounds of muriate of potash.

Kentucky (40): Organic matter and lime of soil should be increased along with the phosphoric acid.

United States Department of Agriculture (49): Fair application of acid phosphate and KCl on soils of low fertility.

Goessman (13), of Massachusetts, as far back as 1892 claimed that manure and sodium nitrate gave better results with soybeans than minerals alone.

Brooks (4), of Massachusetts, reports that the soybean is one of the crops which responds better to sulfate of potash than to the muriate. He also shows that lime in the different forms increases the yields considerably.

Lipman and his associates (31), in pot experiments with soybeans, showed that there was little difference in the crop yield or nitrogen content of the plants fertilized with various amounts of $CaSO_4$, acid phosphate, $NaNO_3$ and $CaCO_3$. The soil used was a loam fairly rich in plant-food, and as the plants were all well inoculated these results were rather to be expected. $CaCO_3$ gave as high nitrogen percentages in the plants as did $NaNO_3$. $CaSO_4$ gave the lowest percentage of nitrogen in the plants. Doubling the amount of $CaCO_3$, $NaNO_3$ or acid phosphate did not affect the composition (protein content) of the plant appreciably, although the yield was increased.

In another place (33) he showed that gypsum had little or no effect upon the yield or protein content of soybeans. In a liming experiment, 1000 pounds was found to be as beneficial as 4000 pounds. A sandy soil appeared to favor a high protein content of soybeans.

Shive (44) working with soybeans in sand cultures, obtained some interesting results. He found an osmotic concentration of salts of 0.05 to 0.1 atmosphere best for soybeans; greater concentrations were injurious. Ammonium salts, with the exception of $(NH_4)_2SO_4$, exerted a more toxic effect on soybeans than any of the corresponding salts of K, Na or Ca. Phosphates

caused injury to most of the seedlings grown in solutions containing the higher concentrations of the radical PO_4. This may account for the injury to germination which has been repeatedly noted, when soybeans are drilled in direct contact with the fertilizer, the latter usually being acid phosphate.

Lipman and Blair (29) showed that the nitrogen content of soybeans increased with applications of $NaNO_3$, $(NH_4)_2SO_4$ and dried blood. In sand cultures they found that nodule development was not depressed by nitrogenous fertilizers. The yield of dry matter increased with the applications of nitrogenous fertilizers up to a maximum, and then decreased. Miss Thompson (46), of the Hawaii Experiment Station, showed that soybeans and other legumes growing in different soil types had varying percentages of nitrogen.

Shedd (43) of the Kentucky Station did much work upon the relation of sulfur to soil fertility. He showed that with soybeans the best results were obtained with sulfur, ammonium sulfate, pyrite and ferrous sulfate. He found that sulfatic fertilizers increased the sulfur content of soybeans, but not necessarily the protein content, as would be expected, since sulfur is a constituent of protein. In two-thirds of the pots, the per cent of protein increases as the sulfur content increases, but not in the same proportion. He showed that ammonium sulfate increased the protein content of soybeans very greatly. In practically all cases sulfur or sulfates gave substantial increases in the dry weight of the soybean plants.

J. K. Wilson (54), in an excellent paper on "Physiological Studies of *Bacillus Radicicola* of Soybeans and of Factors Influencing Nodule Production in Soybeans," points out the effects of a large number of classes of salts on the nodule development. As a general rule chlorides, phosphates, calcium compounds and carbon-containing compounds seemed to stimulate nodule production, while sulfates and ammonia-containing compounds depressed it.

Fertilizers and salts as affecting the oil content of soybean seeds

The researches of Müntz (37), Leclerc du Sablon (26), Gerber (11, 12), and Ivanov (17) with the poppy, flax, sunflower, rape, soybean, castor bean, hemp and sweet almond, all show that the development of oleaginous seeds is characterized by a progressive accumulation of oil accompanied by a corresponding decrease in carbohydrates. This change takes place in unripe seeds detached from the plant, showing quite conclusively that the oil is derived from carbohydrates. Oil accumulation and protein accumulation progress simultaneously, although there is no evidence that there is any relation between the two processes.

Schulze (42) infers that the plant during the period prior to blooming normally accumulates enough nutrients chiefly in the form of carbohydrates and protein to insure the development of the seed. At or near the blooming stage there begins a general movement of simple sugars and soluble nitroggenous constituents through the stem towards the reproductive parts. In the soybeans the oil is classed as "the nitrogen-free reserve food" of the plant.

Garner, Allard and Foubert (10), of the United States Bureau of Plant Industry, late in 1914 made an admirable contribution to the question of the oil content of seeds as affected by the nutrition of the plant. They studied many phases of the question. Some of the deductions which they make as a result of their work are as follows.

In soybeans except for the period immediately following blooming and that directly preceding final maturity, there is a uniform increase in the oil content, both relative and absolute, throughout the development of the seed, and no evidence was found that there is a critical period of very intense oil formation at any stage of the seed development. Maximum oil production requires conditions of nutrition favorable to carbohydrate accumulation during the vegetative period, and to the transformation of carbohydrate into oil during the reproductive period. There is no correlation between size of seed and oil content. No relation was found to exist between the date of planting soybeans and their oil content at maturity, although some varieties shorten the time required to reach maturity if planted late in season. Different varieties of soybeans vary much in the percentage of oil which they contain. Soil type and climatic influences give variable results with soybeans, but as a general rule, they do not greatly affect the oil content. Climate is a more potent factor in influencing the size of the seed and its oil content than soil type. The relative fertility of the soil appears to be a minor factor in influencing the size of the seed and its oil content. Applications of nitrogenous fertilizers to cotton decreased the oil content; no experiments are reported with the soybean. Cylinder experiments with soybeans fertilized with both phosphatic and potassic minerals gave an increase of 20 per cent in the percentage of oil in the seeds.

With phosphorus alone much the same results were obtained, but potash alone had no effect. Peanuts fertilized with phosphorus or potash did not contain more or less oil than plants not fertilized.

Thus we see the status of the effect of various nutritional factors on the yield and oil and protein content of soybeans.

Vegetation experiments to determine the influence of soil texture and fertilizer treatment upon the oil content of soybeans

For this experiment the soils used were Penn fine shaly loam and coarse quartz sand mixed in various proportions as follows:

Pots 1 and 2.	100 per cent Penn fine shaly loam (soil).
Pots 3 and 4.	80 per cent soil and 20 per cent sand.
Pots 5 and 6.	60 per cent soil and 40 per cent sand.
Pots 7 and 8.	40 per cent soil and 60 per cent sand.
Pots 9 and 10.	20 per cent soil and 80 per cent sand.
Pots 11 and 12.	0 per cent soil and 100 per cent sand.

The above is the relative order of the pots in all 8 series. Eight such series of pots were run with various fertilizer treatments. Series I was a check, and no fertilizers were used. Series II was treated with 2 gm. acid phosphate; Series III with 2 gm. $NaNO_3$; Series IV with 2 gm. KCl; Series V with 2 gm. each of acid phosphate and $NaNO_3$; Series VI with 2 gm. each of acid phosphate and KCl; Series VII with 2 gm. each of $NaNO_3$ and KCl, and Series VIII with 2 gm. each of acid phosphate, $NaNO_3$ and KCl.

The glazed earthenware pots contained 9.5 pounds of soil, or soil and sand as the case might be. Black Eyebrow soybeans were planted in each pot and thinned to 7 plants per pot. The pots were kept at optimum moisture content. This was determined for the first two weeks by weighing the pots, but this entailed so much labor that the practice was discontinued. After weighing a few times it was observed that one could easily tell the amount of water needed to bring the soil to optimum very closely. The crop was not all harvested at the same time since some of the plants matured before others. For instance, the pots of Series III, V and VII matured later than the others, because of the nitrogen with which they were fertilized.

To all of the pots 0.25 gm. of $MgSO_4$, 0.25 gm. of $Fe_2(SO_4)_3$ and 5 gm. of $CaCO_3$ were added. All pots were inoculated with soil infusion. The data obtained are given in table 7 (Series I to VIII).

Beginning with table 7, Series I, it is seen that the yield of dry matter in all the pots containing any soil is nearly the same. Pots 3 and 4 are exceptions. Here some unknown harmful factor prevented the plants from growing even after repeated trials. The analysis of the seeds gives but little information of value as to why the pots failed to produce seed. The indications are that a balanced mixture of soil and sand produces higher oil content in the seeds than soil or nearly pure sand. As was to be expected, the pure sand produced but a small crop.

Series II shows an increase in dry matter per pot over Series I. This must be due to the acid phosphate added, as other conditions were the same. The phosphate produced the best results in pots where there was considerable sand mixed with the soil, pointing to a possible better utilization of the phosphorus in sandy soils. As in Series I, the highest oil percentages in the seed are found in soils of medium texture. The general tendency of the results shows that phosphorus slightly increases the oil content of soybean seeds, although the data are not convincing. This may be due to the important rôle phosphorus is supposed to play in seed formation.

The addition of $NaNO_3$ in Series III causes a decrease in both dry-matter and oil content of seeds. This is in keeping with the results of other experiments herein reported. The oil content is decreased from 1 to 2 per cent in nearly all of the pots. The effect of the nitrate was further pointed out by the tall, spindling, weak vines produced by the plants in this series. In the pure sand pots of both Series II and III there is but little plant growth, showing that essential elements are lacking there.

TABLE 7

Effect of fertilizers upon the oil content of soybeans

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	gm.	*gm.*	*per cent*
Series I. No fertilizers			
1	12.0	12.05	18.2
2	12.0		
3*	2.3	1.65	No seed
4*	1.0		
5	9.4	11.10	19.0
6	12.8		
7	12.0	12.60	19.3
8	13.2		
9	11.3	11.85	17.9
10	12.4		
11	6.9	6.85	No seed
12	6.8		
Series II. 2 gm. acid phosphate			
13	12.2	12.50	18.7
14	12.8		
15	12.1	13.60	19.0
16	15.1		
17	14.2	13.50	19.5
18	12.2		
19	13.3	14.90	18.6
20	16.5		
21	10.2	9.55	Too few seeds
22	8.9		
23	7.1	6.65	No seed
24	5.2		

* Some unknown harmful factor caused the plants to die even after repeated plantings.

TABLE 7—*Continued*

Series III. 2 gm. $NaNO_3$ per pot

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	gm.	*gm.*	*per cent*
25	9.4	12.05	17.2
26	14.7		
27	12.6	11.95	17.3
28	11.3		
29	16.0	13.85	17.6
30	11.7		
31	13.3	13.30	18.1
32	13.3		
33	12.0	12.55	18.0
34	13.1		
35	6.0	6.15	No seed
36	6.3		
	Series IV. 2 gm. KCl per pot		
37	13.8	13.30	18.1
38	12.8		
39	12.1	11.95	18.5
40	11.8		
41	7.4	8.05	15.3†
42	8.7		
43	9.8	10.50	17.6†
44	11.2		
45	6.3	7.05	Too little seed
46	7.8		
47	3.0	3.85	No seed
48	4.7		

† Small seeds.

TABLE 7—*Continued*

Series V. 2 gm. acid phosphate + 2 gm. $NaNO_3$

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	gm.	*gm.*	*per cent*
49	16.4	15.15	17.8
50	14.9		
51	15.0	15.30	17.7
52	15.6		
53	15.7	16.10	18.0
54	16.5		
55	16.0	15.90	18.3
56	15.8		
57	13.7	12.75	17.4
58	11.8		
59	8.5	8.25	Too few seeds
60	8.0		

Series VI. 2 gm. acid phosphate + 2 gm. KCl

61	13.3	11.50	18.6
62	9.7		
63	12.8	11.35	18.1
64	9.9		
65	12.0	12.00	18.2
66	12.0		
67	9.3	8.75	16.7†
68	8.2		
69	6.8	6.05	No seeds
70	5.3		
71	3.0	3.45	No seeds
72	3.9		

TABLE 7—*Continued*

Series VII. 2 gm. $NaNO_3$ and 2 gm. KCl

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	gm.	*gm.*	*per cent*
73	12.1	11.55	16.7†
74	11.0		
75	11.7	11.70	17.6†
76			
77	14.4	13.45	17.8
78	12.5		
79	11.4	11.35	17.2†
80	11.3		
81	8.5	7.75	No seed
82	7.0		
83	6.0	5.50	No seed
84	5.0		

Series VIII. 2 gm. acid phosphate, 2 gm. $NaNO_3$ and 2 gm. KCl

85	14.0	14.00	18.10
86	14.0		
87	14.0	14.45	18.30
88	14.9		
89	12.8	12.60	18.40
90	12.4		
91	14.5	13.60	17.60†
92	12.7		
93	9.8	9.40	16.30†
94	9.0		
95	2.6	3.20	No seed
96	3.8		

† Small seeds.

The results of Series IV fertilized with KCl alone are much the same as those of the check Series I, only the yield of dry matter in several of the pots is even less than in the latter series. The per cent of oil in the seeds is variable, but seems to be a little lower in the pots fertilized with potash alone than in Series I and II, where there were no fertilization and 2 gm. of acid phosphate, respectively. The oil content, however, was higher than in Series III, fertilized with $NaNO_3$.

Series V produced the largest yield per pot of any series thus far. The oil content is intermediate between Series II and Series III, as would be expected since both acid phosphate and nitrate of soda are added to each pot in this series.

In Series VI, fertilized with KCl and acid phosphate, the yield of dry matter is lower than in Series II, having acid phosphate alone. Such results cannot be accounted for unless KCl tends to depress the yield, as it apparently did in Series IV. The percentage of oil increases again over Series V, as no $NaNO_3$ is present, and compares favorably with Series II, where only acid phosphate was used. It was noticed that, in some of the pots of all of the series, small seeds occurred in some of the pods. This may have been due to lack of proper nourishment or other causes, but these small seeds always gave a lower percentage of oil than the large well-formed seeds. Oil determinations made from plants bearing many small seeds are marked with a star to call attention to the fact. Such determinations were invariably low.

Series VII, fertilized with both $NaNO_3$ and KCl, gave yields of dry matter slightly lower than Series III, which had $NaNO_3$ only. The oil percentages also are low, attributable no doubt to the soluble nitrogenous salt.

Complete fertilizer was used on Series VIII with the result that good healthy-looking plants were obtained, although the yield of the series as a whole was not as high as of Series V, containing acid phosphate and $NaNO_3$ only. The yield is higher than from any of the other series, however. The oil content also is fairly high but not as high as in a number of other series. Where a complete fertilizer is present the peculiar effect of $NaNO_3$ in lowering the percentage of oil in the seed does not seem to be as noticeable.

Deductions from the vegetation experiments

No practical deductions may be drawn from these vegetation experiments because the results obtained are not sufficiently conclusive, and also because of the variability of the percentage of oil in soybean seeds, obtained from different pots. $NaNO_3$ decreased the oil content of the seeds in every case. Acid phosphate generally increased it slightly or else had no effect. KCl appeared to depress the oil content slightly. Too great concentrations of salts in the sand or near sand pots may account for the stunted plant growth obtained in most of these pots.

After harvesting the crop the soil was well stirred up, and a second crop of soybeans planted. The red spider attacked the young plants with such avidity that the experiment was ruined and discontinued.

Field experiments conducted to determine the effect of various fertilizers upon the yield, nodule production, oil and protein content of soybeans

The plots used in this experiment were located on Penn fine sandy loam. They were 5 by 10 feet in size, comprising 0.001141 acre. The soil was very uniform in fertility as shown by the previous grass crop. All plots were divided from each other by a space of 1 foot. The inoculation used was soil from an old soybean field, applied at the rate of 2 pounds per plot. The infection was very good. A peculiar yet very important observation was made concerning the spread of the nodule-producing bacteria in the soil. Many soybean plants were growing outside of the plots so as to make the end plots of the experiment as nearly as possible like the others, and it was noticed that these plants had either very few nodules or no nodules at all, even though they were growing within a few feet of richly infected soil. The plots were planted to Black Eyebrow soybeans on June 4, 1916, and harvested on September 19, 107 days later. The beans were planted in rows, three rows per plot, and carefully cultivated during the summer. To determine the dry matter produced on each plot, samples of ten plants were carefully weighed, dried, and weighed again. It was then a simple matter to get the dry weight of the total yield of each plot. Of course, the material on each plot was weighed green as harvested, and later again when the beans were flailed out to determine the relative yields of straw and seed. The lime used was ground oyster shells. The lime requirement of the soil as shown by the Veitch method was about 6000 pounds of CaO. The amount of $CaCO_3$ used on the limed plots was 2000 pounds per acre. Various fertilizers and combinations of fertilizers, both with and without lime were used.

The results are given in the following tables.

Table 8 shows that applications of acid phosphate to soybeans give increased yields, provided plenty of lime is present in the soil. Small applications 100 to 200 pounds per acre were as beneficial as greater amounts on limed soil. The yield of seed also was increased by the phosphatic fertilizer. From these experiments it appears that small applications of 100 to 200 pounds of acid phosphate per acre may be profitably used on limed soils, but not on unlimed soils. On acid soils lime is apparently a greater factor than plant-food. It corrects the soil acidity, mellows heavy soils, makes the plant-food in the soil more available to plants by stimulating bacterial activity, and lastly, makes the soil a congenial home for the nitrogen-gathering bacteria. Nodule formation was increased considerably on the limed plots by the acid phosphate, especially in the plots receiving from 100 to 300 pounds per acre; on the unlimed plots there was little or no increase in the

number of nodules per plant where phosphorus was applied. Comparing the limed with the unlimed plots it is seen at a glance that both yield of dry matter and bean seed, and the number of nodules per plant were much increased by the use of lime with the acid phosphate.

TABLE 8

Effect of phosphates on yield and nodule production in soybeans

PLOT	TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	
1	2000 pounds $CaCO_3$	4.90	2.15	2.75	4.93	2.16	0.36	0.30	20.2
1A	100 pounds acid phosphate	4.95	2.16	2.79					
2	2000 pounds $CaCO_3$	5.06	2.06	3.00	4.95	1.98	0.38	0.12	24.8
2A	200 pounds acid phosphate	4.83	1.90	2.93					
3	2000 pounds $CaCO_3$	4.92	1.74	3.18	4.80	1.76	0.27	−0.10	26.4
3A	300 pounds acid phosphate	4.67	1.77	2.90					
4	2000 pounds $CaCO_3$	4.96	2.00	2.96	4.75	1.91	0.18	0.05	18.5
4A	500 pounds acid phosphate	4.53	1.82	2.71					
5	Check	4.59	1.81	2.68	4.57	1.86			18.6
5A	2000 pounds $CaCO_3$	4.54	1.91	2.63					
6	No lime	4.34	1.57	2.77	4.12	1.59	0.24	0.23	17.4
6A	100 pounds acid phosphate	3.89	1.61	2.28					
7	No lime	3.63	1.32	2.31	3.67	1.39	−0.21	0.03	21.3
7A	200 pounds acid phosphate	3.70	1.45	2.25					
8	No lime	3.54	1.12	2.42	3.47	1.26	−0.41	−0.10	13.1
8A	300 pounds acid phosphate	3.40	1.37	2.03					
9	No lime	3.42	1.10	2.32	3.43	1.18	−0.45	−0.18	14.6
9A	500 pounds acid phosphate	3.43	1.25	2.18					
10	Check	3.93	1.43	2.40	3.88	1.36			14.8
10A	No lime, no acid phosphate	3.83	1.29	2.64					

The use of acid phosphate with lime seemed to cause a small yet consistent increase in the oil content of beans over the check plot (table 9). In the case of the unlimed plots phosphorus did not raise the percentage of oil in the seed. As regards the protein content, little or no influence of phos-

phorus is noted on the limed plots, but on the unlimed there seems to be a small increase in the percentage of protein in the seed due to the application of acid phosphate. Since phosphorus is a constituent of protein, one would rather expect heavy applications of phosphatic fertilizers to increase the percentage of the former in the seed, but this does not necessarily hold true, since many other limiting factors may enter into the problem.

TABLE 9

Effect of phosphates on the oil and protein content of soybeans

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	17.8	42.5	17.50	42.60	1.26	−0.05
1A	17.2	42.7				
2	16.9	42.0	17.00	42.00	0.75	−0.65
2A	17.1	42.0				
3	17.6	41.5	16.85	42.40	0.60	−0.25
3A	16.1	43.3				
4	16.3	43.3	16.30	42.55	0.05	−0.10
4A	16.3	41.8				
5	16.6	42.6	16.25	42.65		
5A	15.9	42.7				
6	18.1	39.2	17.90	39.60	−1.25	1.30
6A	17.7	40.0				
7	19.4	39.0	19.40	38.40	0.75	0.10
7A	19.4	37.8				
8	17.8	38.6	18.15	39.45	−0.50	1.15
8A	18.5	40.3				
9	17.9	38.9	18.60	39.25	−0.05	0.95
9A	19.3	39.6				
10	18.6	38.0	18.65	38.30		
10A	18.7	38.6				

Potash, although an essential element to plant growth, does not seem to be needed as much on most soils as phosphorus or nitrogen. On the Penn loam, however, where this series of experiments was conducted, it gave increased yields of dry matter and seed over the checks, in all applications from 50 to 400 pounds per acre (table 10). This increased yield took place on both limed and unlimed plots. The increase was approximately 10 per cent,

hence from a practical point of view it would pay to make a small application of potash to soybeans. Small applications gave practically the same results, or even better results than higher amounts. Potash stimulated

TABLE 10

Effect of potash on the yield and nodule production of soybeans

PLOT NO.	FERTILIZER TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
11	50 pounds KCl	4.89	2.00	2.89	4.86	1.98	0.53	0.10	25.4
11A	2000 pounds $CaCO_3$	4.83	1.95	2.88					
12	100 pounds KCl	4.84	2.04	2.80	4.75	2.00	0.52	0.12	25.7
12A	2000 pounds $CaCO_3$	4.66	1.97	2.69					
13	200 pounds KCl	4.40	1.95	2.45	4.52	1.93	0.19	0.05	23.8
13A	2000 pounds $CaCO_3$	4.65	1.90	2.75					
14	400 pounds KCl	4.33	2.05	2.28	4.64	2.09	0.31	0.11	31.4
14A	2000 pounds $CaCO_3$	4.94	2.13	2.81					
15	Check	3.88	1.86	2.02	4.33	1.88			21.2
15A	2000 pounds $CaCO_3$	4.77	1.90	2.87					
16	50 pounds KCl	4.91	1.80	3.11	4.87	1.85	0.87	0.19	19.0
16A		4.83	1.90	2.93					
17	100 pounds KCl	4.68	2.00	2.68	4.63	1.96	0.63	0.30	20.2
17A		4.58	1.93	2.55					
18A	200 pounds KCl	4.08	1.56	2.52	4.12	1.60	0.12	−0.06	27.9
18A		4.16	1.64	2.46					
19	400 pounds KCl	4.08	1.65	2.43	4.20	1.68	0.20	0.02	16.9
19A		4.31	1.71	2.60					
20	Check	3.99	1.71	2.28	4.00	1.66			20.9
20A		4.02	1.60	2.42					

nodule production slightly on the limed plots, but no differences were obtained on the unlimed plots. Here again the yield of dry matter and seeds was considerably greater on the limed plots.

Potash appears to decrease slightly the oil content of soybean seeds in all the plots except one (table 11). The same is true of the percentage of pro-

tein, although the differences are almost negligible. On the limed plots the oil content is considerably lower than on the unlimed plots.

For calculating the increase in yield over the check in this experiment it was deemed better to use as the check the average of 6 plots, namely, 5 and 5A, 15, 15A, 28 and 28A than to use only plots 28 and 28A, included prop-

TABLE 11

Effect of potash on the oil and protein content of soybeans

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
11	17.5	39.7	17.0	39.0	−1.8	−4.4
11A	16.5	40.8				
12	17.5	43.0	17.9	43.3	−0.9	−0.1
12A	18.2	43.5				
13	17.5	42.0	17.7	41.3	−1.1	−2.1
13A	17.9	40.5				
14	15.7	41.4	15.9	41.3	−2.9	−2.1
14A	16.0	41.1				
15	17.4	43.0	18.8	43.4		
15A	20.1	43.8				
16	18.7	39.9	18.7	39.7	−0.63	−0.0
16A	17.6	39.4				
17	18.4	39.2	17.8	39.7	−1.1	0.0
17A	17.3	40.2				
18	19.8	38.7	19.6	39.0	−1.7	−0.7
18A	19.3	39.2				
19	18.5	38.4	18.1	38.8	−0.8	−0.9
19A	19.6	39.2				
20	18.3	39.8	18.9	39.7		
20A	19.4	39.6				

erly in this experiment. As the soil is very uniform it is permissible to do this, and thus secure more reliable data. All of the plots received 2000 pounds of ground oyster shells in addition to the fertilizer mixtures as given in the tables. Because of an error in fertilizing, no check plot was left at plots 24 and 24A, where one would naturally have occurred according to the original scheme of the experiment.

All of the fertilizer mixtures gave increases over the check plots in the yield of dry matter, and except for plots 27 and 27X, treated with manganese, in seed also (table 12). The most efficient combination of fertilizers seemed to be that combining nitrogen, phosphorus and potash. These combinations make up a complete fertilizer, and gave the largest increases over the check, as a general thing. Whether such a fertilizer is economical or not is a different matter. It is likely that the greatest returns for money invested would be obtained by using lime and acid phosphate alone. The fact that plot 23 had 200 pounds per acre more potash than plot 22 does not seem to affect the yield favorably.

However, plot 21, with only 100 pounds per acre of potash, gives as great a yield as plot 23, with 400 pounds. From these results it would seem to indicate that small applications of potash up to 100 pounds per acre might pay on soils like the one where the experiment was carried on. Nitrate of soda produced increased dry matter and seed yields, showing that where soluble nitrogenous plant-food is readily accessible to the soybean plant it is absorbed and used by the latter. This is not an economical way of supplying plant-food, however, since it has been demonstrated that legumes are able to obtain the greater part of their nitrogen from the atmosphere. The presence of 200 pounds per acre of nitrate of soda very seriously interferes with nodule formation, as is shown by plot 26. This plot produced plants having on an average 15.2 nodules, while the check plot produced about 26. Nodule formation was not notably depressed by the other combination of fertilizer used. Wherever nitrate was used a slight reduction in the number of nodules per plant was noticed. As shown before, phosphorus and potash have a stimulating action upon nodule formation.

The use of manganese sulfate as a fertilizer or chemical stimulant has been advocated by many men, and has been used in this way on various crops with widely different results. As much manganese ore refuse is easily obtained at the zinc mines in northern New Jersey, it was decided to try its effect upon the growth of soybeans. The data show that a benefit is derived from the use of 50, 100 and 500 pounds per acre of $MnSO_4$. The yield of seed is not increased except on plot 31 with an application of 500 pounds per acre. The increases are rather small, so it is not likely that it would pay to apply manganese-containing substances as a fertilizer. Besides, the beneficial results obtained from the use of $MnSO_4$ may come from the SO_4 radical and not from the Mn. That it does stimulate germination and early growth of soybeans is apparently true, as the manganese-treated plots were up a number of days before any of the others. The early growth was also stimulated but the other plots soon equaled the manganese-treated plots in height, and many passed them within two weeks from the date of planting. There is little to indicate that nodule production is greatly affected by applications of manganese salts; if anything they have a slightly depressing effect.

TABLE 12

Effect of various fertilizers on the yield and nodule production of soybeans

PLOT NO.	FERTILIZER TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK†	INCREASE IN SEED OVER CHECK†	NUMBER OF NODULES PER PLANT
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
21	Acid phosphate 300 pounds KCl 100 pounds	5.23	2.08	3.15	5.43	2.09	0.92	0.23	28.1
21A	$CaCO_3$ 2000 pounds	5.63	2.10	3.53					
22	Acid phosphate 300 pounds KCl 200 pounds	4.16*	1.90	2.26	4.71	1.95	0.20	0.09	28.7
22A	$CaCO_3$ 2000 pounds	5.25	1.99	3.26					
23	Acid phosphate 300 pounds KCl 400 pounds	5.41	2.06	3.35	5.41	2.08	0.90	0.22	34.9
23A	$CaCO_3$ 2000 pounds	5.40	2.09	3.31					
24	Acid phosphate 300 pounds $CaCO_3$ 2000 pounds	5.88	2.12	3.76	6.36	2.14	1.85	0.28	26.6
24A	KCl none	6.84	2.17	4.67					
25	Acid phosphate 300 pounds KCl 200 pounds	6.03	2.24	3.79	6.11	2.20	1.60	0.34	24.7
25A	$CaCO_3$ 2000 pounds $NaNO_3$ 100 pounds	6.18	2.16	4.02					
26	Acid phosphate 300 pounds KCl 200 pounds	6.22	2.34	3.88	6.25	2.32	1.74	0.46	15.2
26A	$CaCO_3$ 2000 pounds $NaNO_3$ 200 pounds	6.27	2.29	3.98					
27	$MnSO_4$ 100 pounds	5.03	1.45	3.58	4.97	1.70	0.46	−0.14	23.5
27A	$CaCO_3$ 2000 pounds	4.90	1.94	2.96					
27X	$MnSO_4$ 50 pounds	4.52	1.62	2.90	4.71	1.82	0.20	−0.04	23.5
27AX	$CaCO_3$ 2000 pounds	5.06	2.02	3.04					
28	Check	4.14	1.67	2.47	4.63	1.84			26.1
28A	$CaCO_3$ 2000 pounds	5.13	2.00	3.23					
29	KCl 200 pounds $NaNO_3$ 200 pounds	4.53	1.85	2.68	4.63	1.98	0.12	0.12	25.9
29A	$CaCO_3$ 2000 pounds	4.72	2.10	2.72					
30	KCl none	4.83	1.87	2.96	5.51	2.02	1.00	0.16	23.8
30A	$NaNO_3$ 200 pounds $CaCO_3$ 2000 pounds	6.18	2.16	4.02					
31	$MnSO_4$ 500 pounds $CaCO_3$ 2000 pounds	5.00	2.11	2.89	5.00	2.11	0.49	0.25	

* Sample for moisture determination partly eaten by rats.

† The average value of check plots No. *5*, No. *15* and No. *28* is meant here.

In regard to the effect of fertilizer mixtures upon the oil and protein content of soybean seeds, the data are conflicting and not convincing (table 13). $MnSO_4$ in all three plots greatly depressed the percentage of protein in the beans, and two of the three plots gave an increase in oil content over the check.

TABLE 13

Effect of various fertilizers on the oil and protein content of soybeans

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
21	19.9	43.5	19.65	43.65	1.68	0.82
21A	19.4	43.8				
22	16.5	43.8	16.10	43.40	−1.87	0.57
22A	15.7	43.0				
23	18.7	42.8	19.00	43.05	0.65	0.22
23A	19.3	43.3				
24	17.2	43.5	17.15	43.40	−0.82	0.57
24A	17.1	43.3				
25	17.4	45.3	18.15	44.75	0.18	1.92
25A	18.9	44.2				
26	19.0	44.0	18.50	43.65	0.53	0.82
26A	18.0	43.3				
27	18.3	40.1	18.15	40.45	0.18	−2.38
27A	18.0	40.0				
27X	17.8	41.5	18.55	42.25	−0.58	−0.58
27AX	19.3	43.0				
28	19.1	42.0	18.85	42.45		
28A	18.6	42.9				
29	17.9	42.0	17.35	42.65	−0.62	−0.38
29A	16.8	43.3				
30	18.9	41.4	18.9	42.30	0.93	−0.53
30A	18.9	43.2				
31	19.4	41.1	19.4	41.10	1.43	−1.73

Phosphorus and potash, as in other experiments herein reported, appear to increase the protein content slightly, but the results with the oil are too variable to warrant the drawing of even a tentative conclusion. Possibly one reason for the failure to obtain greater differences in crop yield and com-

position was the fact that all the plots were limed, thus liberating sufficient plant-food for the crop's needs, as well as stimulating the nodule bacteria and a greater fixation of nitrogen.

Field experiments on the effect of sulfur, sulfates, and nitrates upon the yield, oil, and protein content of soybeans

For this experiment plots were laid out on acid Sassafras sandy loam on land adjoining the lime and inoculation experiments already discussed. The plots were laid out exactly the same as in previous experiments, except for the fertilizer treatment. This consisted of Basic slag, 400 pounds per acre; muriate of potash, 200 pounds; and ground oyster shells, 2000 pounds. Besides this general treatment the plots were fertilized with special applications of sulfur, sulfates and nitrates. All of the plots were inoculated by spreading about 2 pounds of inoculated soil on each plot. Black Eyebrow soybeans were planted July 1 and harvested on October 2. The A plots were of slightly higher fertility than the duplicates, as the result of a heavier sod in places, but the soil as a general thing was fairly uniform. The native vegetation on the soil previous to plowing showed it to be of poor fertility. The results obtained are given in tables 14 and 15.

Elemental sulfur did not give increased yields of seed in these experiments in quantities over 200 pounds per acre. An application of 200 pounds per acre resulted in a slightly increased yield of seed, but there is no doubt that on this soil, greater amounts are injurious to soybeans. The yield of dry matter was noticeably lower on the plots having applications of 400 and 600 pounds per acre than on the check plots. Unfortunately, these data were lost and we have only the yield of seed as a criterion.

From the data it appears that the protein content of the seeds is increased by a moderate application of sulfur, but decreased by larger amounts. That sulfur in quantities over 200 pounds per acre interfered with the normal growth of the plants was readily observed throughout the experiment. The plants were not healthy or leafy, but seemed to produce considerable seed in spite of this. Since sulfur is a constituent of protein it seems reasonable that moderate amounts would increase the percentage of protein in the seeds. This was found to be the tendency of the results obtained by Shedd (43), of the Kentucky Experiment Station. However, he analyzed the immature plants and not the seeds.

The oil content of soybean seeds, contrary to the results obtained for protein, is decreased by moderate applications of sulfur and increased by the larger ones. In the seed obtained from plot 102, where sulfur at the rate of 400 pounds per acre was applied, the increase in the content of oil was 1.4 per cent.

Nodule production is probably somewhat stimulated by small applications of sulfur. The data are not conclusive, because of the wide variation between checks.

Calcium sulfate or gypsum was found to exert little influence on the yield of seed until 600 pounds per acre had been applied, when a small increase in the seed yield was noted. As in the case with free sulfur, the oil content was raised and the protein content of the seeds lowered, with large applications (600 pounds) of land plaster per acre. Calcium sulfate caused an ap-

TABLE 14

Effect of sulfur and gypsum on the yield and composition of soybeans

PLOT NO.	FERTILIZER TREATMENT	SEED	OIL	PROTEIN	AVERAGE SEED	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN SEED OVER CHECK	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	*per cent*	*per cent*	*lbs.*	*per cent*	*per cent*	*lbs.*	*per cent*	*per cent*	
100	Check	0.87	19.5	40.3	0.98	19.8	39.5				4.8
100A		1.08	20.2	38.6							
101	200 pounds sulfur	1.03	16.8	40.2	1.08	17.4	40.3	+0.15	−0.80	+0.6	7.7
101A		1.12	17.8	40.4							
102	400 pounds sulfur	0.87	19.9	40.3	0.88	19.6	39.6	−0.05	+1.40	−0.1	11.6
102A		0.88	19.2	38.8							
103	600 pounds sulfur	0.71	18.0	36.1	0.73	18.4	43.4	−0.20	+0.2	−2.3	6.0
103A		0.74	18.7	38.6							
104	Check	0.89	16.3	40.9	0.88	16.5	39.8				11.2
104A		0.86	16.6	38.7							
105	200 pounds $CaSO_4$	0.87	17.3	41.7	0.87	17.4	40.9	−0.17	−1.1	+0.2	15.4
105A		0.87	17.5	40.0							
106	400 pounds $CaSO_4$	0.98	17.2	40.3	0.98	17.5	38.9	−0.06	−1.0	−1.8	19.7
106A		0.98	17.8	37.6							
107	600 pounds $CaSO_4$	1.27	19.3	38.6	1.34	19.7	38.6	+0.30	+1.2	−2.1	20.9
107A		1.41	20.1	38.6							
108	Check	1.17	20.1	42.0	1.19	20.4	41.5				12.9
108A		1.20	20.7	41.0							

parent increase in nodule production in all the plots where it was used. This is in accord with the work of Wilson (54), of Cornell, who found it depressed nodule formation in young soybean plants. He found also that $MnSO_4$ stimulated nodule production, while $ZnSO_4$ and iron tersulfate depressed it. In the present experiment it was found that, if anything, $MnSO_4$ slightly depressed the number of nodules per plant, while $ZnSO_4$ and $F_2(SO_4)_3$ caused a

still greater depression. All of the sodium-nitrate-fertilized plots caused a very marked decrease in the number of nodules per plant.

Concerning the yield of seed on the $MnSO_4$, $ZnSO_4$, and $Fe_2(SO_4)_3$ plots the data show a slight increase for the latter two plots, but for the plots

TABLE 15

Effect of sulfates and nitrates on yield and composition of soybeans

PLOT NO.	FERTILIZER TREATMENT	SEED	OIL	PROTEIN	AVERAGE SEED	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN SEED OVER CHECK	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	*per cent*	*per cent*	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
109	$NaNO_3$	1.39	19.7	40.6	1.29	20.2	40.0	0.08	+0.8	−0.7	20.7
109A	100 pounds	1.19	20.7	39.9							
110	$NaNO_3$	1.57	20.8	39.6	1.58	20.8	39.8	+0.21	+1.4	−0.9	18.5
110A	200 pounds	1.58	20.7	39.6							
111	$NaNO_3$	1.60	18.4	43.3	1.58	18.2	43.3	+0.21	−1.2	+2.6	16.0
111A	600 pounds	1.56	18.0	43.3							
112	Check	1.53	18.1	40.3	1.55	18.3	39.9				28.3
112A		1.56	18.4	39.5							
113	$NaNO_3$	1.37	18.3	42.4	1.52	18.7	42.2	−0.19	0.0	+1.4	18.3
113A	400 pounds	1.67	19.1	42.0							
114	$MnSO_4$	1.43	19.2	39.9	1.62	19.8	39.6	−0.09	+1.1	−1.2	16.0
114A	50 pounds	1.81	20.3	39.2							
115	$MnSO_4$	1.17	16.7	40.1	1.26	17.3	40.6	−0.45	−1.4	−0.2	24.6
115A	100 pounds	1.35	17.8	41.0							
116	$ZnSO_4$	1.54	18.0	42.7	1.37	18.2	42.6	+0.16	−0.5	+2.2	21.2
116A	100 pounds	2.20	18.3	42.4							
117	Check	1.76	19.0	41.0	1.86	19.1	41.8				27.0
117A		1.96	19.2	42.5							
118	$FeSO_4$	2.54	18.4	41.3	2.54	18.4	41.8	+0.68	−0.7	0.0	16.2
118A	100 pounds	2.54	18.4	42.3							

fertilized with 50 and 100 pounds of $MnSO_4$ per acre there was a decrease. This is in accord with other experiments reported in this paper, where $MnSO_4$ caused a small decrease in drop yield on Penn loam soil; zinc and iron sulfates slightly increased the oil content of the soybeans produced on these plots, the former causing an increase of 2.2 per cent in protein over checks

and the latter no increase or decrease over checks. $MnSO_4$ increased the protein content of beans from both plots where it was used, but the oil content is increased by an application of 50 pounds per acre and decreased by an application of double this amount. Such results mean nothing, and conclusions drawn from them, unless the experiments have been repeated a number of times, are worthless.

Sodium nitrate gave variable results in regard to the yield of seed. It is certain, however, that it would not pay to use it as a fertilizer for soybeans on this type of soil. Large applications, 400 and 600 pounds per acre, increased the protein content of the seeds appreciably, but the use of smaller amounts did not. Small amounts of nitrate on this soil gave an actual increase in oil content of the soybeans, but an application of 400 pounds per acre gave no increase, and one of 600 pounds per acre gave a decrease of 1.2 per cent.

SUMMARY AND PRACTICAL DEDUCTIONS

From both vegetation and field experiments, it was found that certain commercial cultures of bacteria for inoculating soybeans are not reliable, while others are as efficient in producing nodules in the host plant as freshly-isolated cultures of *B. radicicola* or well infected soil. Inoculation gave a substantial increase in the yield of total dry matter and of seed of soybeans in every case. An average decrease of 3 per cent in the oil content of soybean seeds was caused by inoculation. The protein content, on the contrary, was increased 7 per cent. The oil content is decreased and the protein content increased in direct proportion to the thoroughness of infection of the plants. No differences in the drying power of the oil extracted from the seeds of inoculated and uninoculated plants was observed. Natural inoculation of soil sometimes spreads very slowly, as it was repeatedly observed that plants on inoculated plats at a distance of a foot or two from uninoculated plats, were seldom found inoculated. It appears that unless *B. radicicola* is transferred by means of winds, water, animals, etc., its progress is very slow in the soil.

Ground oyster shells and burnt lime were very efficient in increasing the yield and total dry matter of soybeans on acid soils; the increase varying from 30 to 50 per cent. Small applications (1000 to 2000 pounds per acre) are nearly as beneficial as large amounts, and are, of course, much more economical. Small applications of lime at intervals of a few years are to be preferred to a single large application. It appears that liming soybeans on acid soils is nearly as important as inoculation. Both should be practised together for the best results. On sour soils liming stimulated nodule production to a marked degree—in some cases as much as 1500 per cent. The bacterial infection of roots does not take place readily on acid soils even when the root-infecting organisms are plentiful in the soil. The oil content of soybeans decreases in direct proportion to the largeness of applications of lime

applied to the soil; conversely, the protein increases. The average decrease in oil content due to liming was 2.8 per cent. Small amounts of lime are nearly as efficient in raising the protein content of soybean seeds as larger applications.

Immature and small seeds are lower in oil content than mature seeds. This may be explained by assuming that reserve carbohydrates in the seed have not become fully transformed into oil.

The yield of total dry matter and seed of soybeans is materially increased by small applications of acid phosphate, especially when the soils are well limed. One to two hundred pounds per acre appears to be as beneficial as large applications. On acid soils, acid phosphate did not give any appreciable increase, hence the soil should be first limed before applying phosphatic fertilizers. Nodule production on soybeans was also stimulated on limed soils by acid phosphate but this was not so marked on acid soils. Oil production in the seeds was increased on the limed plots but not on the unlimed. Acid phosphate, however, seems to exert a beneficial influence on protein formation in the seed on both limed and unlimed plots.

Potash (muriate), in applications of from 50 to 400 pounds per acre, gave an average increase of about 10 per cent in the yield of total dry matter and seed on both limed and unlimed plots. Nodule production was slightly stimulated on the limed plots, but not on the unlimed. Potash caused a slight decrease in the percentage of oil in the seeds, but had little influence on their protein content.

Various combinations of acid phosphate, muriate of potash and nitrate of soda, with a dressing of lime, all gave substantial increases in the yield of total dry matter and except for two plots fertilized with manganese sulfate, in seed as well. That fertilizer treatment which would appear to give the greatest return for the money invested on acid soils, with soybeans as the crop, is probably 200 to 300 pounds of acid phosphate together with a ton of lime. Other fertilizer mixtures give increases in the crop, but they are not sufficient to justify using them. Nitrate of soda, for example, increased the yield but inhibited nodule formation and consequent fixation of atmospheric nitrogen. It is not economical to supply soluble plant-food in the form of nitrogenous fertilizers to soybeans. Nitrate of soda caused an appreciable increase in the protein content of soybean seeds, and also a decrease in their oil content.

Manganese sulfate stimulated germination and early growth of soybeans but did not stimulate nodule production nor give increased yields. It had little, if any, effect upon the oil or protein content of the seed.

Elemental sulfur did not give increased yields of dry matter or seed in applications over 100 pounds per acre. Larger amounts seemed to injure the plants. Perhaps this was due to the oxidation of the sulfur in the soil to H_2SO_4, thus producing acidity. It appears that the protein content of soybeans is increased by moderate applications of sulfur, but is decreased by

large applications. The exact reverse is true in the case of oil content. In general, sulfur stimulates nodule formation.

Land plaster in amounts up to 600 pounds per acre exerted little influence on the yield of total dry matter or seed. Large amounts caused an increase in oil content in the seed and also stimulated nodule formation.

The results on the plots where zinc sulfate and ferric sulfate were used are not conclusive, but these minerals seemed to stimulate the growth of the plants and gave increased seed production. The protein content also was somewhat increased. The oil content was slightly decreased.

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PLATE 1

EFFECT OF INOCULATION ON THE SIZE AND LEAFINESS OF SOYBEANS


INOCULATED
NO INOCULATION

PLATE 2

EFFECT OF LIMING ON THE ROOT DEVELOPMENT AND NUMBER OF NODULES

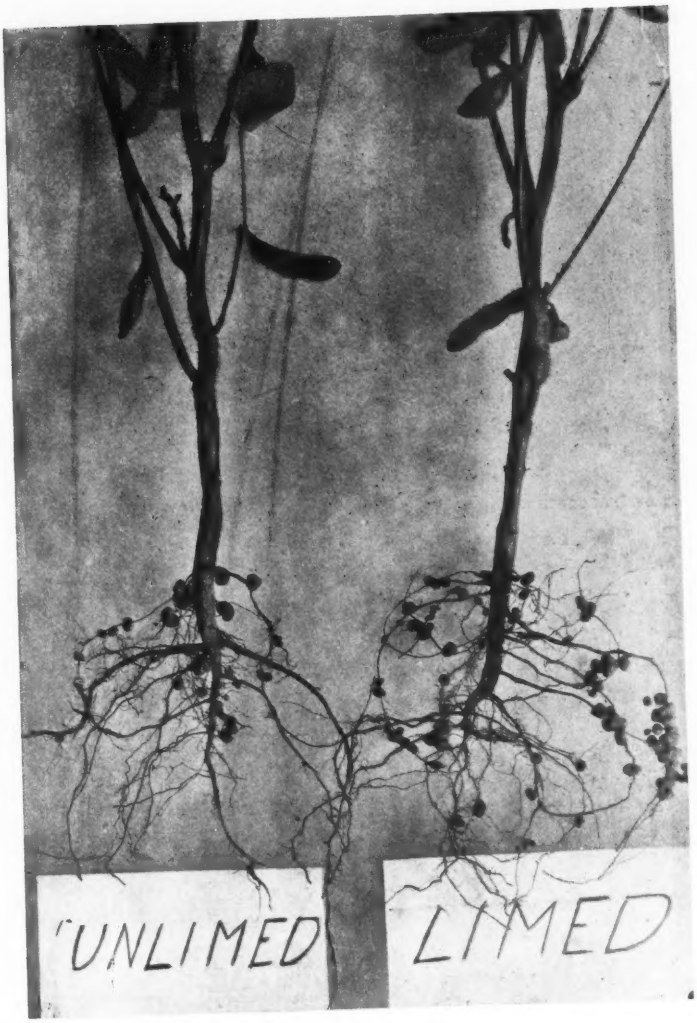

UNLIMED
LIMED

PLATE 3

EFFECT OF INOCULATION ON THE SIZE OF THE SOYBEAN PLANT AND ITS ROOT DEVELOPMENT

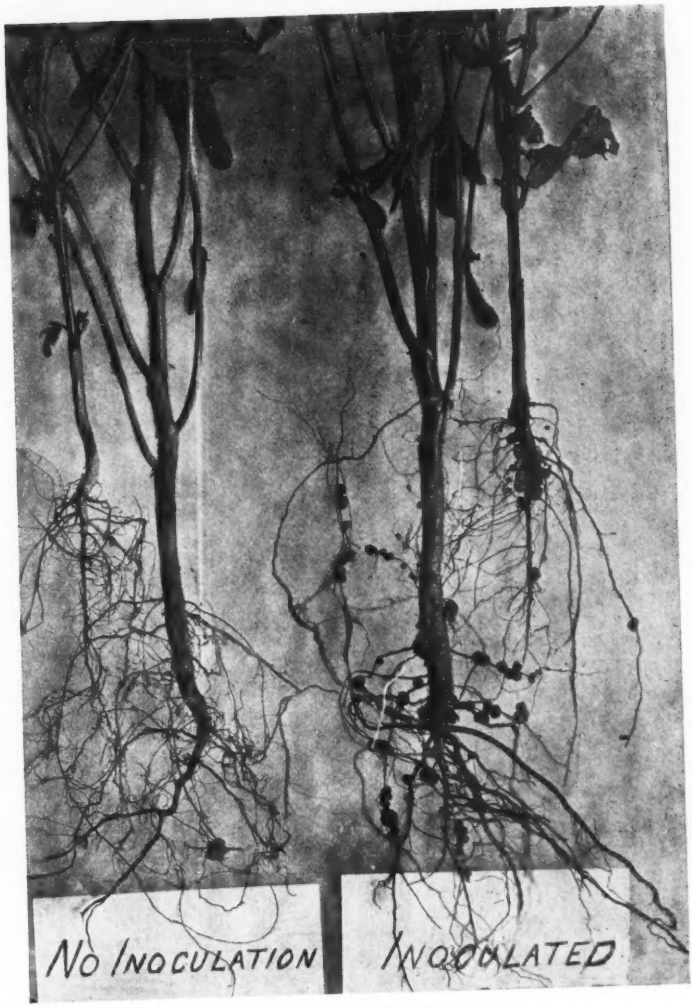

NO INOCULATION
INOCULATED

PLATE 4

EFFECT OF $NaNO_3$ ON THE SIZE AND NODULE FORMATION IN SOYBEANS

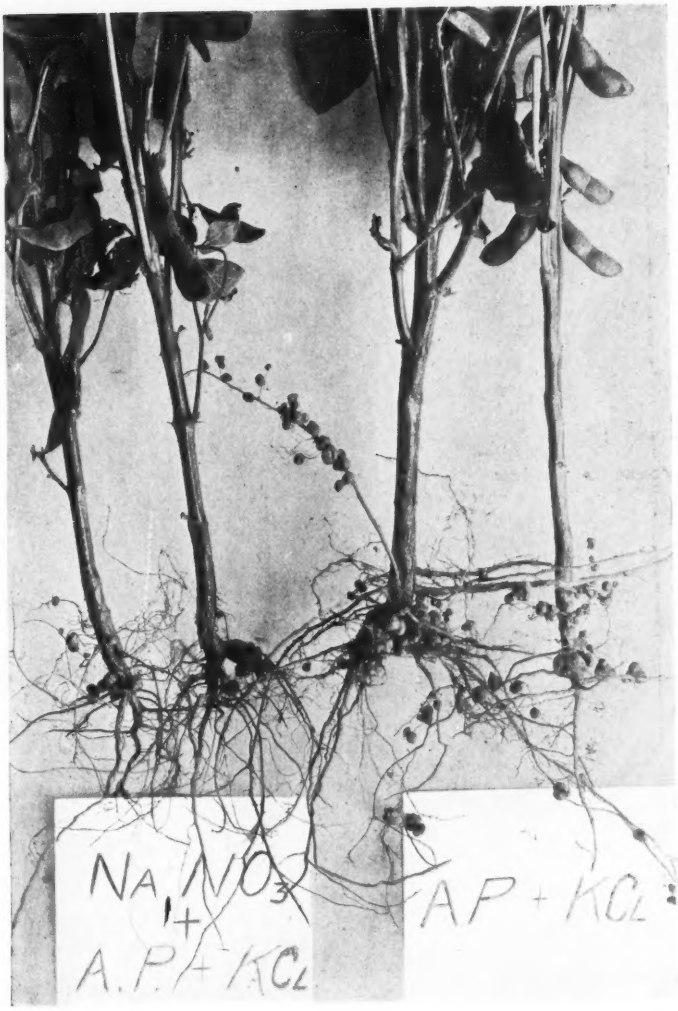
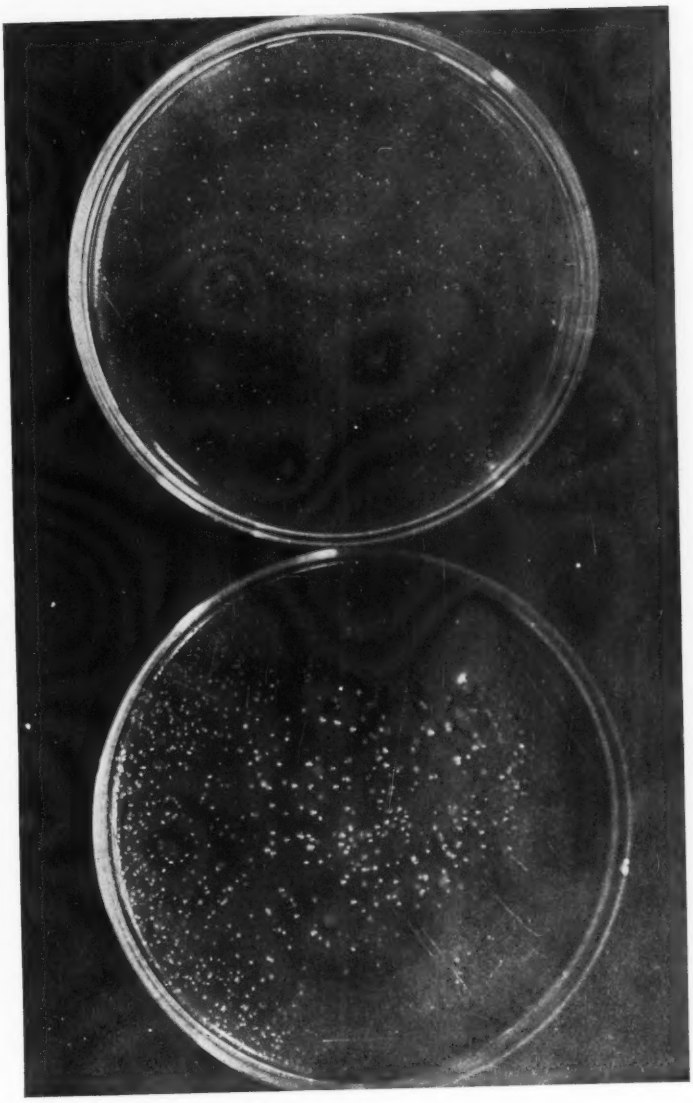

NA NO3
+
A.P.+KCL
AP + KCL

PLATE 5

COLONIES OF *B. radicicola* 10 DAYS OLD ON ASHBY'S MEDIUM SHOWING THE DIFFERENCES IN SIZE OF THE COLONIES PRODUCED BY THE SOYBEAN (UPPER) AND THE ALFALFA (LOWER) BACTERIA



ARE UNUSUAL PRECAUTIONS NECESSARY IN TAKING SOIL SAMPLES FOR ORDINARY BACTERIOLOGICAL TESTS?

C. B. LIPMAN AND D. E. MARTIN

University of California, Agricultural Experiment Station, Berkeley, California

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The method of obtaining soil samples for bacteriological study has formed the subject of many investigations since the time of the introduction by Robert Koch of the gelatin plate method into bacteriological technique. In all such studies, the underlying idea has been to guard against the contamination of the samples by dust or by soil from adjoining layers, each of which was to be examined separately. It seems, however, that such contamination was assumed to be appreciable without actual knowledge regarding its extent and its significance. So far as we are aware, no experiments have been conducted to determine the real status of the factor of contamination in soil sampling. In connection with other studies on the "soil columns" of California which were carried out jointly by Hilgard, Loughridge, and the senior author of this paper, the assumption above mentioned was also made when the bacteriological studies were planned and executed. The unusual precautions taken in sampling at that time were emphasized a number of years ago in a paper by C. B. Lipman (1). In planning recently a continuation of the aforementioned bacteriological work, however, it occurred to the senior author that much time, labor and expense might be saved in connection with the necessary soil sampling if the extreme precautions taken in the earlier work could be obviated, by reason of the knowledge that the contamination due to sampling with an auger was an insignificant factor in the work. The idea that the contamination resulting from auger sampling was probably slight seemed to be supported by the logical reasoning that the magnitude and biochemical efficacy of soil flora in a given sample could not readily be influenced by the addition of relatively small numbers of organisms from adjoining soils.

In view of these considerations, further studies of the flora of additional soil columns, to determine the depths below the surface to which bacteria penetrate and are active, were suspended until a proper comparison of sampling with the auger and sampling with unusual precautions from a vertical wall could be carried out. Two soils were chosen for the experiment, one an alluvial loam at Hayward, the other a blow sand at Oakley. The tests used as criteria in this experiment were the following: bacterial counts on bouillon agar, ammonifying power with 0.1 per cent peptone, nitrifying power with soil nitrogen alone, and with soil nitrogen plus sulfate of ammonia (0.2 per cent), and nitrogen fixation in solutions with 2 per cent mannite and in soil with 1 per cent mannite.

The sampling was done as follows: A hole was dug to a depth of 5 feet, and having one vertical wall. The wall was sterilized by a thorough flaming with a plumber's torch. Examination showed that the soil was dried and charred to a depth of $\frac{1}{2}$ inch. Then, starting at the fifth foot, the flamed surface was scraped away to a depth of 1 inch by a downward cut of a sterile spatula thus laying bare fresh soil. This was then sampled by the resterilized spatula for the total length of the foot. The soil was put directly into previously sterilized cotton-stoppered, glass Mason jars. Each foot was sampled by the same process successively from the fifth foot to the first, the spatula, of course, being resterilized for every foot sampled. The reason for starting with the fifth foot and progressing upward is, of course, obvious, the lowest depth being the region of least bacterial activity. Then, also, soil dislodged and falling down during sampling would not contaminate surfaces to be sampled. The auger samples to be compared with these were taken by boring, with slight precautions, into the soil adjacent to the wall (about 3 inches from it) from which the first set of samples just described were obtained. The auger used was of one of the post-hole type manufactured by Iwan Brothers at South Bend, Indiana. The samples were placed as rapidly as taken into sterile glass fruit jars, cotton-stoppered, and sent to the laboratory for study. The results of the experiment are given in the subjoined tables. Samples 1 to 5, inclusive, are those taken by the sterile spatula from the vertical wall and are marked "aseptic." Samples 6 to 10 are those taken by the auger and are so marked in the tables. For convenience, we shall discuss briefly each set of tests by itself.

THE BACTERIAL COUNTS

Considering the appreciable error which inheres in methods of making bacterial counts, it is surprising to find how well the data for both methods of taking the samples in the cases of both soils agree (table 1). The variations which do occur are sometimes in one direction and sometimes in another and in general are too small to be significant. Incidentally, the counts gave a good picture of the numbers of the bacterial population at the different depths in the two soils, and of the relative paucity in bacterial numbers which characterizes the poorer as against the richer soil.

AMMONIFICATION DATA

The ammonification results show tendencies similar to those of the bacterial counts (table 2). Again the first foot shows itself to be markedly superior to the lower depths in the Hayward soil, but not so in the Oakley soil. Below the first foot in the Hayward soil and at all depths in the Oakley soil, the ammonifying power seems to be about uniform down to the sixth foot. This is all incidental, however, to the main question at issue here, which seems to be answered unequivocally. The results of the auger and those of the "aseptic" method run practically parallel and more distinctly so than those of the bacterial counts among themselves.

NITRIFICATION DATA

Fully as striking as the ammonification data, if not more so, are the nitrification results (table 3). For all practical purposes, the two methods of sampling appear to yield identical figures on the nitrifying power of the soil for its own

TABLE 1

Bacterial counts

Bouillon Agar—incubation one week at 28°C.

METHOD	NUMBER	KIND OF SOIL	
		Hayward	Oakley
		Organisms per gram	*Organisms per gram*
Aseptic	1	100,000,000	2,760,000
	2	65,000,000	1,750,000
	3	5,000,000	1,300,000
	4	5,200,200	1,700,000
	5	4,000,000	860,000
Auger	6	130,000,000	2,500,000
	7	30,000,000	1,900,000
	8	4,400,000	1,450,000
	9	5,400,000	1,400,000
	10	3,000,000	900,000

TABLE 2

Ammonification

0.1 per cent Peptone—50 grams soil—incubation one week at 28°C.

METHOD	NUMBER	AMMONIA NITROGEN PRODUCED	
		Hayward soil	Oakley soil
		mgm.	*mgm.*
Aseptic	1	17.78	8.26
	2	8.12	9.52
	3	7.28	9.66
	4	5.30	9.66
	5	5.30	8.82
Auger	6	18.20	8.40
	7	7.70	9.38
	8	6.86	9.24
	9	6.02	8.68
	10	5.32	8.82

nitrogen as well as for that of sulfate of ammonia. This is true for both soils. Incidentally, again, it is interesting to note the superiority of the first foot in this case of both soils as against the lower depths. The nitrifying powers of the

Hayward soil stand out in sharp contrast, however, with the ammonifying powers of the same soil as above determined. The successive depths decrease rapidly in their power to produce nitrate, but they do not do so as regards ammonia production, except as between the first foot and all the others which appear to be about uniform. In the Oakley soil, on the other hand, the nitrification data are very similar in general trend to the ammonification data, except that in the case of the sulfate of ammonia nitrogen, the first foot of soil, behaves, in general, like that of the Hayward soil.

NITROGEN-FIXATION DATA

The only significant data, in one sense, which have been adduced from the nitrogen-fixation studies are contained in the results for the Hayward soils (table 4). These seem to indicate, both in the solution and in the soil cultures, that there is no appreciable contamination of one layer of soil by another when the samples are taken by means of the auger. In the latter respect, however, even the data for the Oakley soil, while showing little or no fixation of nitrogen, are significant, since the samples taken by the auger yielded virtually the same results as those taken "aseptically." On the other hand, it is obvious that not much contamination of one layer of soil by another with nitrogen-fixing bacteria could be induced through the auger method of sampling when no nitrogen-fixing bacteria were present. There having been no *Azotobacter* membranes observed in the mannite solution, cultures with the Oakley soil, except as noted in two cultures which probably became contaminated, it is not surprising that little or no nitrogen fixation was obtained. Absolutely none was obtained in the soil cultures and less than half a milligram in every case in the solution cultures. This discrepancy and other circumstances discussed render it probable that the data for the soil cultures reflect more correctly the status of the Oakley soil with respect to nitrogen fixation.

In the Hayward soil, *Azotobacter* membranes were found in all of the solution cultures, and were about of equal vigor for the cultures prepared from the auger and the "aseptically" collected samples. In the solution cultures, nitrogen fixation seemed to be most vigorous in the first 3 feet and less so in the last 2 feet. In the soil cultures, however, nitrogen fixation was very marked in the first 2 feet and fell off sharply in the other 3 feet in which it appeared to be uniform. There was general agreement in this respect between the cultures made from the two sets of samples tested.

SUMMARY AND CONCLUSIONS

It seems clear from the foregoing tables and discussions that there is no necessity for taking unusual precautions in sampling soils for bacteriological work. This is at least true for all such ordinary tests as are made in most laboratories to obtain certain bacterial coefficients of soils. The ordinary removal of samples from the field soil by augers, with slight precaution, seems to intro-

duce no contamination from one depth of soil to another, as the auger passes downward. Tests for bacterial counts, ammonification, nitrification, and nitrogen fixation on soil columns 5 feet in depth give strong evidence in support of the foregoing assertion. The reason for the evident failure of the auger method of sampling to affect the soil from layer to layer by direct contamination is probably to be found in the inadequacy of the relatively small numbers of organisms introduced, to the task of affecting to a perceptible degree the much larger and more firmly established flora already existing in a given soil stratum. The investigators, including the senior author, who have attempted to devise methods for preventing contamination of soil samples by taking unusual pre-

TABLE 3

Nitrification

METHOD	NUMBER	NITROGEN AS NITRATE PRODUCED FROM TWO SOURCES OF NITROGEN					
		Hayward soil			Oakley soil		
		Nitrate in soil initially	Nitrate produced from soil nitrogen	Nitrate produced from ammonium sulfate	Nitrate in soil initially	Nitrate produced from soil nitrogen	Nitrate produced from ammonium sulfate
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Aseptic	1	0.35	1.50	10.00	Trace	0.25	1.00
	2	Trace	1.00	2.75	Trace	0.20	0.25
	3		0.35	1.50	Trace	0.25	0.25
	4		0.25	0.75	Trace	0.25	0.25
	5		0.35	0.75	Trace	0.40	0.35
Auger	6	0.35	1.50	10.00	Trace	0.25	0.80
	7		1.00	2.50	Trace	0.30	0.45
	8		0.35	1.50	Trace	0.15	0.30
	9		0.25	0.75	Trace	0.25	0.15
	10		0.35	0.80	Trace	0.30	0.25

cautions, have based their efforts on what seems now to be an erroneous assumption. The latter was in turn due to a lack of knowledge as to whether or not contamination in ordinary soil bacteriological work is a factor of significance. From the experiments which we have carried out in a study of this question, the following conclusions, among others, have resulted:

1. For ordinary bacteriological work on soils, no special precautions are necessary in taking soil samples.

2. Samples taken to a depth of 5 feet each with an ordinary soil auger, and by the "aseptic" method from a flamed vertical wall of a pit, respectively, show no significant dissimilarity in bacterial counts, ammonifying power for peptone (0.1 per cent in soil), nitrifying power, and nitrogen-fixing power. That the sampling itself from the vertical wall constituted a "precautionary" method is indicated by the striking contrasts which characterize the comparisons made above in various respects of the different layers of a given soil.

3. All attempts at devising methods of soil sampling for ordinary soil bacteriological work have been based evidently upon the erroneous assumption that dangers from contamination in such work are considerable.

4. The soil flora in a given sample of soil seem to be so large, so characteristic, and so firmly established and adapted to the conditions under which they are found that the introduction of relatively small numbers of contaminating organisms into that sample are without perceptible effect on the original flora as shown in ordinary tests on soils.

5. Incidentally to the main conclusions of this paper, it is also to be noted that in the two soils studied, as in others described in a paper above cited, large

TABLE 4

Nitrogen fixation

METHOD	NUMBER	NITROGEN FIXATION IN SOLUTION 50 CC. MANNITE SOLUTION—INCUBATION 2 WEEKS AT 28°C		NITROGEN FIXATION IN SOIL 100 GM. SOIL + 1 PER CENT MANNITE—INCUBATION 3 WEEKS AT 28°C	
		NITROGEN FIXED PER GRAM MANNITE		NITROGEN FIXED PER GRAM MANNITE	
		Hayward soil	Oakley soil	Hayward soil	Oakley soil
		mgm.	*mgm.*	*mgm.*	*mgm.*
Aseptic	1	10.99	0.57	14.00	0.00
	2	9.31	0.49	19.60	0.00
	3	8.47	0.49	5.60	0.00
	4	4.20	0.57	4.20	0.00
	5	5.32	1.54*	7.00	0.00
Auger	6	10.08	0.57	16.80	0.00
	7	7.49	0.57	21.00	0.00
	8	7.35	0.77	5.60	0.00
	9	6.51	1.12*	7.00	0.00
	10	5.32	0.84	8.40	0.00

*Probably contaminated. *Azotobacter* film.

bacterial numbers and notable bacterial activity are to be found at relatively great depths in soils of the arid region. This probably is very different from the conditions which characterize soils of the humid region.

6. Despite the evidence mentioned in the foregoing conclusion, respecting the great depths to which bacteria penetrate in arid soils, it is to be noted that in practically all tests, the surface foot of soil is by far the most active bacteriochemically speaking, and is by far the most densely populated. In some phases of the soil's bacterial activity, however, the second foot approaches or equals the first foot. As a rule, the soil layers from 2 feet down to 6 feet are nearly uniform in bacterial population and activity in a given soil.

REFERENCE

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THE IMPORTANCE OF MOLD ACTION IN THE SOIL[1]

SELMAN A. WAKSMAN

Department of Soil Bacteriology, New Jersey Agricultural Experiment Station

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INTRODUCTION

When a group of microörganisms is studied in relation to soil fertility, the first question that presents itself is: What is the nitrogen metabolism of these organisms? What part do they play in the nitrogen cycle of the soil? The largest number of investigations dealing with soil microörganisms, whether in pure or in mixed cultures, whether studied in solution or in the soil, in the laboratory or in the field, are concerned with the possible nitrogen changes in the soil produced as a result of the activities of these organisms. Since the nitrogen changes in the soil are among the most important ones in the study of soil fertility, the wildest speculations have often been made in an attempt to interpret the nitrogen changes produced in the laboratory upon artificial culture media from a few organic or inorganic substances by microörganisms and account thus for the different soil processes which are important from the point of view of soil fertility problems. Fewer investigations were devoted to the study of the carbon, phosphorus, iron, sulfur, and potash changes in the soil as a result of microbial activity.

The very methods employed in soil bacteriological investigation can be subjected to a great deal of criticism. Without going at present into a detailed discussion of the different methods used in the study of nitrogen metabolism of the soil flora as a whole, an attempt will be made in this paper to take up the study of the metabolism of molds which commonly occur in the soil. This may help to throw some light upon the part played by these organisms in different soil biological processes.

From the early period of soil microbial investigations up to the last four or five years, nearly all the attention of the soil bacteriologists was centered upon the study of bacteria, entirely neglecting the other groups of microörganisms, although these must have come to their attention here and there. The fact could not be overlooked that the soil has an abundance of molds, actinomycetes, protozoa, rotifers and, under certain conditions, algae, which exist in the soil in an active state. Whether the cause for neglect was the use of media more favorable to bacteria than to the other microörganisms for their isolation from the soil; whether it was due to the fact that the bacteria form the

[1] The species of fungi isolated from the soil belong to widely scattered groups; the common term "mold" is applied here collectively to these organisms, although no sharp limitation can be placed on the use of the term.

more numerous group of soil organisms, and, as always happens in such cases, the attention is centered upon the more frequently-occurring types; whether the important advance along bacteriological lines from the point of view of pathogenicity to men and animals has centered upon it so much of the attention even of the soil bacteriologist, that the other organisms (non-bacterial) were neglected—whichever was the cause, it was only within the last half-dozen years that the great abundance of other microörganisms in the soil besides bacteria, has been demonstrated and an attempt has been made to explain their probable part in soil fertility.

The work of Russell and his associates (48) upon the possible part played by protozoa in soil fertility has called forth a series of investigations on the occurrence of soil protozoa and their activities. The algae of the soil were studied by several investigators. The occurrence of actinomycetes and their possible rôle in the soil have been recently summarized in several papers. It will be the object of this paper to point out several metabolic processes of the molds occurring in the soil and thus attempt a suggestion as to their part in soil fertility. An extensive bibliography on this subject can be found elsewhere (56). The several soil biological processes will be taken up and, by comparing the activities of the molds with those of bacteria, the important transformations which the organic matter and the mineral matter undergo in the soil and which may be due to the action of the molds, will be discussed.

The observations and conclusions are based on the work of different investigators, but chiefly on that of the writer.

OCCURRENCE OF MOLDS IN THE SOIL

It has been definitely established (56, 58) that the molds are common inhabitants of the soil and form a large and important group of the soil flora.

Hundreds of species of molds have been isolated repeatedly from the soil; it has been found that many molds occur in different soils, under different topographic, climatic and crop conditions. The same species have been isolated by a number of investigators in different European countries and from numerous soils in this country. A number of new species never encountered before were isolated from the soil, which would serve as additional proof that some of these are typical soil organisms. Although as many as 1,000,000 fungus colonies developed from 1 gm. of soil in some cases, this cannot be taken as proof that so many pieces of mycelium were present in that quantity of soil, but merely that a mass of spores were present and each of these spores developed into a colony when the soil was shaken with water and plated out. The method of determining the number of molds in the soil is very inefficient, and since usually only two or three plates are made from each dilution, the probable error is much greater than anything that should be allowed for exact work. This was the reason why the writer, in one of the earlier publications (58), attached little importance to the plate count of molds in the

soil. And before more exact work has been done along this line and a large enough number of samples and soils have been studied, we shall not be able to state definitely as to how many molds (or rather mold spores and pieces of mycelium) can be expected in each gram of soil; even then, the uncertainty will exist because of the fact that at one spot an organism might have sporulated, which would give a tremendously large number of spores, not indicating, however, any predominant rôle for this organism over others which may occur in much smaller numbers. For example, such organisms as the Aspergilli or Penicillia, which produce a very large number of spores separating with relative ease, are often found in predominant numbers, while at a different period of the year these organisms may be entirely absent.

This may account for the statement of Hagem (20) that certain Aspergilli occur in larger numbers in the soil than all the Mucors taken together. This statement was made for Norway soils, and as the writer pointed out elsewhere (58), the Aspergilli are more abundant in a warmer climate, while the colder climates show a greater proportion of Mucorales in the soil.

A detailed discussion as to the occurrence of the different genera and species of fungi in different soils, as well as the methods of isolation, both in vegetative mycelial and spore forms can be found elsewhere (56, 58). In reviewing the work of others and his own, the writer concluded that there is a fungus flora of the soil and that many molds occurring in one soil will also be found in other soils, under different conditions of cultivation topography, origin and climate. The fact was pointed out that the Northern soils have a greater abundance of Mucorales and Penicillia, while the Southern soils are richer in Aspergilli; Trichodermae are found in large numbers in acid soils; Fusaria, Cladosporia, Chaetomia, Alternaria, etc. will be always found in most soils, if a large enough number of samples are studied and care taken in isolation. The list will no doubt be extended a great deal with a more intimate knowledge of the different more or less obscure forms of fungi present in the soil which require the careful study of the specialist in the different groups of these organisms.

Ramann (44) found that molds develop readily in acid soils and are more active in the forest and in the compact poor soils, while bacteria predominate in loose soils, rich in nutrients, cultivated and fertilized. Hagem (20) stated that in the well cultivated lands containing little humus the bacteria play an important part and occur in predominant numbers and the molds are of minor importance; while the upper layers of pine forests, rich in humus, contain a large number of molds. The rainy seasons of the year, fall especially, favor the surface growth of these organisms, otherwise they live and sporulate below the surface, among the plant residues and living roots. Since the last publication of the writer (58) there appeared a paper by Pratt, who isolated from Idaho soils a number of organisms, particularly Mucors, Fusaria and Penicillia, many of which were isolated by the writer previously from different soils of North America.

The fact that molds are found in the soil would not warrant as yet any extensive study of these organisms, before it has actually been demonstrated that they are not only present in the soil, but actually live there and produce mycelium, which would necessitate their taking an active part in the different biological transformations taking place in the soil. The writer (57) suggested a method by which it could be demonstrated that molds actually live in the soil, and, although not all the organisms found in the soil could be demonstrated by this method to have produced mycelium (curiously enough, the organisms found in the largest numbers, such as Aspergilli and Penicillia, could be demonstrated in the soil by this method only in very few cases), a number of groups were found to be present in the soil in an active state.

Conn (9), using a direct microscopic method for examining soils, found that mold mycelium is present in the soil only to a very limited extent. If plate counts of molds present in the soil do not have the same significance as plate counts of bacteria, as pointed out by Conn (9) who was preceded in this by the writer (58, p. 571), the microscopic method of Conn (9) is probably of not much greater value in this respect. The relative numbers of spores and hyphae of typical soil molds is such that, as Conn himself states, 3000 microscopic fields would have to be examined before a piece of mycelium could be found, while enough spores were produced by the same organisms to give a plate count of 300,000 per gram of the soil in which it would be grown.

Brown (4) repeated the work of both the writer and Conn and demonstrated that mycelium was present in all soils examined. Conn, using very small quantities of soils, namely 10 mgm., could easily have overlooked the mycelium that might have been present, although Brown stated that by using the writer's method he could demonstrate mold mycelium not only in 10 mgm. but also in smaller quantities of soil. Thom and Church (53) observed that different Aspergilli and Penicillia planted upon the surface of sterilized soil are capable of growing into the soil to considerable depths and even of producing spores.

The fact should not be overlooked that most of the investigations upon soil molds were made by botanists, who were either interested more in the types of the organisms as such, or as to their possible pathogenicity to plants, but not from the point of view of soil fertility. To be able to interpret the proper part played by these organisms in the soil we must study them not only as vegetative or other forms of plants, but as living organisms which exist in the soil, and by their metabolic processes help in the various transformations through which both organic and inorganic soil constituents undergo and thus affect the fertility of the soil.

The universal presence of molds in the air, on decaying fruit and different forms of organic matter, has brought them early to the attention of the biochemist, who used some of these organisms in investigations on metabolism. But since the fact has been established that these organisms exist in the soil, their biochemical activities could then be interpreted so as to understand their possible part in the fertility of the soil.

NITROGEN-FIXATION

The question of nitrogen-fixation by molds commonly isolated from the soil was under dispute, some investigators demonstrating that it was positive, while others could not find any fixation at all. The very recent work of Goddard (19), Waksman (56), Duggar and Davis (17) and Chambers (7), using more exact chemical methods and taking all precautions to eliminate any possible error that may creep into such experiments, has shown that the fungi commonly occurring in the soil, such as the different species of Aspergilli, Penicillia, Macrosporia, Alternaria, Mucors and others do not fix any atmospheric nitrogen. The amount of nitrogen-fixation by molds claimed to be positive by certain recent workers is so slight (below 5 mgm. per 50 cc. of solution) as to be of practically no importance in soil fertility when compared with the nitrogen-fixing bacteria. The slight quantities of nitrogen-fixation obtained by several workers may possibly be explained by the fact that no precautions were taken to eliminate any nitrogen compounds from the air of the laboratory, to have the chemicals carefully analyzed and to make a large enough series of determinations so as to eliminate any possible error. A detailed discussion of the literature on the subject can be found in the papers of the writers mentioned above. The mycorhiza fungi, as shown recently by Peklo (39) and certain other fungi, not commonly occurring in the soil, such as cultures of *Phoma Betae*, studied by Duggar and Davis (17), may show a definite nitrogen-fixation.

It can therefore be definitely concluded that, with the exception of certain organisms, which are not very common in the soil, the typical soil molds do not play any direct part in the economy of the nitrogen enrichment of the soils.

NITRIFICATION

Neither nitrite nor nitrate formation has ever been demonstrated for any of the molds, so that this important process in soil fertility will have to be eliminated, as well as the nitrogen-fixation, from the field of mold activities in the soil. As will be demonstrated later, the nitrogen activity of the molds tends rather to a process of reduction than to oxidation. The breaking down of the complex proteins to proteoses and peptones, then to polypeptides and amino acids, and finally to ammonia, is accomplished by means of molds just as rapidly and thoroughly as by means of protein-decomposing bacteria. Whether this is accomplished as a result of the feeding of the organism, or by means of enzymes, and also whether it is due to the action of the organism upon the nitrogen part of the protein molecule or its carbon part, will be taken up later. All that can be pointed out here is that the organism decomposes the proteins of the soil with the liberation of ammonia, which is either left in the soil or absorbed by the organism in the process of the building up of microbial proteins. There is no need for the mold to oxidize the ammonia

into nitrates, since the ammonia as such is just as good a form of nitrogen for molds as nitrates are, when other conditions are equal.

Schloesing and Müntz (51) showed in 1878 that *Aspergillus niger* and other molds do not nitrify. The molds may exert an indirect effect upon nitrification, due to the fact that in acid soils the normal bacterial activities may be repressed and the growth of molds encouraged, as pointed out by Hall and his associates (21).

AMMONIFICATION

When we approach the subject of the disintegration of organic matter in the soil, particularly the first stages of decay, the molds are found to play a very important part: both the disintegration of nitrogenous organic compounds, which will be considered under the above heading and the decomposition of celluloses, hemicelluloses and starches in the soil taken up later.

The subject of ammonification has been often misunderstood and such importance attached to the information obtained from the determination of ammonia in the soil that could not possibly be warranted at all by the data. It has not been even definitely settled as yet as to what part ammonia plays in the metabolism of microörganisms. Czapek (12), in an exhaustive study of the utilization of different substances as sources of nitrogen by molds, found that amino acids are used much more economically than other nitrogen compounds. He assumed that molds, in building up their proteins from simple nitrogen compounds such as ammonia salts, have to produce first amino acids, and these are used as building-stones for the production of complex proteins; if amino acids are offered as sources of nitrogen, the organism will be spared the waste of energy which would be necessary if it had to build up its proteins from simpler nitrogenous substances. Hagem (20) claimed that the utilization of amino acids by molds takes place in the following manner. By the addition of water to the amino acid, ammonia and the corresponding oxy-acid are produced; and the protein synthesis takes place from the ammonia thus formed.

Whatever may be the process of formation of complex proteins by molds one thing is certain—that ammonia is left in the medium as a waste product of the protein metabolism of the organism. But even assuming that, we cannot state definitely that ammonia produced by a certain organism or a mixture of organisms will point to a definite condition and interpretation, because the ammonia accumulated as a result of the action of one organism from one nitrogen compound will depend not only on the source of nitrogen, but also on the amount and availability of carbon compounds present in the medium. When we therefore claim that a certain form of life or a complex mixture of different forms, such as a soil flora, will produce so much ammonia from peptone, dried blood, cottonseed meal or other organic nitrogen compounds, and try to interpret from it the activities of the given flora, we may be entirely wrong even if the ammonia produced could be taken as a factor

of the metabolism of the organisms, because the amount of ammonia produced may depend more on the character of the carbon compounds present than on the source of nitrogen. Hagem (20) has shown that although certain amino acids can be used both as sources of energy and nitrogen by molds, the ammonia produced will depend on the presence of carbohydrates in the medium. Kendall and his associates (26) have studied in detail the protective action exerted by available carbohydrates upon the decomposition of proteins by microörganisms. The writer (59, 60), in taking up the same work and applying it to the study of molds isolated from the soil, has tried to develop the theory further and apply it to the changes of the organic matter in the soil due to the action of microörganisms. The bacteria and molds will attack the protein molecule in the soil to derive from it the nitrogen needed for structural purposes, if available carbohydrates are present to supply the energy required; only small quantities of ammonia will be liberated under these conditions. But, in the absence of available carbohydrates, the organisms will attack the protein molecule not only as a source of nitrogen, but also as a source of energy; since the energy requirement of the organism is greater than the nitrogen need, only a small part of the nitrogen of the protein molecule will be used for the building up of the proteins of the microorganism, and the greater part of the nitrogen will be left in the medium as a waste product in the form of ammonia. We should therefore take up the study of ammonification by molds with this idea in mind: the ammonia merely indicates the activities of the organism in the presence of a certain source of carbon and nitrogen. It will indicate, as Hagem (20) stated, the mineralization of the organic matter.

The early workers on ammonification studies used both molds and bacteria. Müntz and Coudon (36) and Marchal (32) demonstrated in 1893 the fact that soil molds are as active ammonifying organisms as bacteria. Marchal (32) even attempted to ascribe the ammonia production in soils (particularly acid soils) chiefly to the action of molds. But following the work of these investigators, the greatest attention has been paid to soil bacteria. Only here and there a paper appeared calling attention to the occurrence of molds in the soils or to the chemical changes produced by them when grown on artificial culture media. Butkewitch (6) observed that in the decomposition of proteins by molds, ammonia and amino acids were formed, among the latter tyrosin and leucin being identified. He has been able to show that, by changing the cultural conditions of the same organism, either a rapid transformation of the peptone into ammonia with the production of only a small quantity of amino acids, or a slow accumulation of ammonia with a large quantity of amino acids takes place. The accumulation of ammonia runs parallel with that of oxalic acid; when the latter is neutralized by means of $CaCO_3$, the ammonia accumulation is suppressed and other nitrogen compounds such as tyrosin and leucin, appear in the substratum. He agrees with Czapek, that amino acids are used directly by the organisms, while ammonia is a by-product

in the decomposition of the complex nitrogen compounds. The least energy was spent by the organism in utilizing amino acids, more energy was used when ammonium salts and nitrates were offered as a source of nitrogen, and still more when peptone and egg-albumen were the nitrogen sources.

Iwanow (23) has shown that, in the decomposition of the seeds of yellow lupins by *A. niger*, small quantities of tyrosin, larger amounts of leucin and still more ammonia, in the form of ammonium oxalate, were produced. Kosyachenko (31) found among the products of decomposition of the proteins of peas by the same organism tyrosin; leucin; the hexone bases histidine, arginine, and lysin; and ammonia. Hagem (20) has shown that, in the utilization of amino acids and peptone by Mucors, ammonia is always produced. Kappen (24) found that several molds decompose cyanamide with the production of ammonia. Other references to the work on the decomposition of organic matter by molds and liberation of ammonia will be found elsewhere (56). McLean and Wilson (34) found that soil molds produce in pure culture a much greater accumulation of ammonia than bacteria. Waksman and Cook (62) confirmed these observations and called attention to the fact that the accumulation of ammonia as a result of mold activities seems to take place in definite cycles and expressed the idea that there may be a relationship between the growth period of the organism and ammonia accumulation. Further information on the subject of decomposition of organic matter by fungi isolated from the soil can be found in the work of the writer (56), who has shown that the growth of molds on artificial culture media affects the ability to decompose organic matter by some molds and not by others. Kopeloff (28) found that an increase in the number of mold spores inoculated into the soil is responsible for a proportionate increase in ammonia production up to a certain point; additional information on the environmental factors influencing the decomposition of organic matter added to sterilized soil by soil fungi are supplied by Coleman (8). The decomposition of urea, uric and bipuric acids and glycocoll by molds with the subsequent liberation of ammonia was studied by Kossovicz (30).

Although, as shown above, the production of ammonia from organic matter, particularly when this has been added to sterilized soil, making the conditions distinctly different from normal, cannot be taken as a true indication of the rôle of these organisms in soil fertility, we are still able to obtain some information from these studies. In nearly all cases reported, some molds, such as *Trichoderma Koningi*, *Monilia sitophila*, and others are able to decompose the organic matter much more rapidly than the strongest ammonifying bacteria known, such as *Bacillus mycoides* or *Bacillus subtilis*. Even if the ammonia is looked upon only as an indication of the amount of organic matter decomposed by the organism, some of the molds, commonly occurring in the soil, are found to possess distinctly greater powers of decomposing the organic matter than do the bacteria. The writer (60) has shown that *A. niger* grown in culture media containing peptone or asparagine as a source

of nitrogen will bring about a rapid decomposition of the material, although the total ammonia production will depend entirely on the amount of available carbohydrate present. It was also pointed out that the ammonia production by *A. niger* is an autocatalytic phenomenon. A word should be said here that, although it has been definitely established by Miyaki (35) for bacteria and by the writer (60) for molds that the ammonia production obeys the law of autocatalysis, as shown by a mathematical interpretation of the results, it has not been demonstrated as yet that a catalyst actually exists, or that the action and counteraction combining to give the curve of autocatalysis may not be merely a result of the growth of the organism (action) and the injurious action of some of the by-products in the culture medium (reaction).

The action of the molds upon the nitrogenous organic matter of the soil consists, therefore, in the mineralization of these materials, with the production of ammonia and the building up of fungus proteins. The ammonia is either used by the higher plants as such or oxidized by the nitrifying bacteria into nitrates and used by the plants in this form, or absorbed again by the microörganisms of the soil either in the form of ammonia or even as nitrates and used for the production of the complex microbial proteins, as will be shown later.

DECOMPOSITION OF CARBON COMPOUNDS IN THE SOIL

The molds play an active part in the decomposition of celluloses and other carbon compounds in the soil; the importance of this process is well known to the student of soils, since the addition of green and animal manures, plant roots and other residues, necessitates this process before the minerals and nitrogen compounds can be brought to a form, in which they could be either taken up by the higher plants directly or after they have undergone another transformation due to the action of other groups of molds or bacteria. Celluloses, pectins, vegetable gums and other similar substances are rather inert and cannot be attacked by all microörganisms harbored in the soil; among those that are able to do it, the molds occupy a prominent place.

The work of Van Iterson (55), Koning (27), Dascewska (13), McBeth and Scales (33), and others has established the fact that the rôle of molds in the soil in the destruction of cellulose has been greatly underestimated; a number of molds were isolated from soil which decompose cellulose very rapidly. The different molds differ greatly in this respect: while some, such as different Penicillia, Aspergilli, Trichodermae, and others dissolve the cellulose very rapidly, others, such as the Mucorales (20) cannot attack it at all. Schellenberg (50) demonstrated that the ability of the molds to dissolve different celluloses does not depend on the solubility of these in acids, but on the chemical composition of the substance. Hagem (20) found that the Mucorales can use, out of all the carbon compounds found in the soil, only the pectin bodies and the mono-saccharides, and partly the disaccharides, while the cellu-

loses and hemicelluloses are left intact. He concluded that the Mucorales must take only a limited part in the decomposition of carbon compounds in the soil, when compared with the other molds and bacteria. Out of nearly a hundred organisms tested by the writer for the ability to decompose cellulose according to the method of McBeth and Scales (33), only the Mucorales and a few other organisms (several Fusaria and Sporotricha) produced little or no decomposition of the cellulose, while the Trichodermae, Cephalosporia, Aspergilli, Penicillia, Verticillia, and others produced a strong or very strong decomposition. A detailed discussion of the action of different compounds would be here out of place; it may only be stated that nearly all the simple and complex organic carbon compounds in the soil can be attacked by one or another group of soil molds, and these, through this action, play an important part in the fertility of the soil.

The question of the humin substances of the soil may be brought up here. Ramann (44) states that Nageli, Hoppe-Seyler, Kostytschew, Müller and others concluded that the molds are the proper humus builders in the soil. The fallen leaves, at the end of the vegetative period in the fall, are found to be penetrated with mold mycelium, which decomposes the leaves readily, with the production of humic substances. Hoppe-Seyler (22) claimed in 1889 that no plant or animal is able to use humin substances as food, and no bacterium can bring forth their decomposition; they afford an habitat and substratum to many bacteria, molds, algae and animals. Reinitzer (45) and Nikitinsky (38) have shown that humin substances cannot serve for molds and bacteria both as a source of carbon and nitrogen, but in the presence of available sources of carbon, they can be used as a source of nitrogen. The value of the study of humin substances in the soil and the action of microörganisms upon them has to be proven as yet, since they are artificial in nature, and the fact has not been even established as yet that they exist as such in the soil, and are not merely decomposition products due to the action of chemicals upon the soil.

As to the utilization of starch and production of amylases, the molds seem to be very active. *Aspergillus Oryzae*, *Aspergillus niger* and other Aspergilli and Penicillia have been found to produce very active starch-splitting enzymes.

The production of carbon dioxide can be taken as an index of the decomposition of soil organic matter. It is a much more accurate index of the biological transformations going on in the soil, since once it is liberated, it is not utilized again by the soil microörganisms, while ammonia and nitrates which are used more commonly by the soil bacteriologists, as indicating soil biological processes, can be utilized again by other organisms in the soil. Neller (38) made recently some interesting observations on the correlation between the production of carbon dioxide and accumulation of ammonia by soil organisms. The molds tested oxidized more of the carbon and produced less ammonia than the bacteria did; mixed soil infusions resembled the molds in the low accumulation of ammonia, but produced larger quantities of carbon

dioxide. Neller, therefore, suggested that the soil molds were the more active components of the natural soil flora, and a low accumulation of ammonia with alfalfa as the source of carbon, may not necessarily indicate a low activity, contrary to the usual conclusions of soil bacteriologists, who took only ammonification, nitrification, or bacterial numbers, as an indication of soil biological processes. A very active production of carbon dioxide from organic matter by molds isolated from the soil was made recently also by Potter and Snyder (40), who have demonstrated that several fungi isolated from the soil liberated nearly as much carbon dioxide from sterilized soil as did a mixed soil flora (soil infusion), both in the presence and absence of dextrose.

These two observations together with the work of the writer (59, 60) on the action of available carbohydrates upon the ammonia production by molds will help to throw a great deal of light upon the activities of these organisms in the soil. The molds attack the carbohydrates very readily, perhaps even more readily, concluding from these few observations, than the bacteria do. This rapid decomposition of the carbohydrates, both complex and simple, indicates a strong activity and rapid need and utilization of energy; the index of the rapid respiration is the carbon dioxide production. The nitrogenous organic compounds may be decomposed only to a smaller extent than they would be in the absence of these available carbohydrates; very little ammonia is therefore produced. But even when the nitrogenous compounds are decomposed rapidly and when a large amount of ammonia would be expected as a waste product of protein metabolism of the organisms, the ammonia will not be accumulated in the medium, but will be used, in its turn, by the organism for the further building up of mold protein, as long as carbohydrates are available to supply the energy. The ammonia will therefore not accumulate in the soil from the nitrogenous compounds, in the presence of available carbohydrates, for two reasons: first, less of the nitrogenous substances will be decomposed and, therefore, less ammonia will be left as a waste product, as shown by the writer (59, 60); second, the ammonia will be further utilized by the organism in the building up of the fungus protein, because of its rapid growth, as shown by the carbon-dioxide production; this can be readily seen from the data obtained by Neller (37). Of course, more information is necessary before we may be able to construct an exact theory as to chemical changes taking place in the soil and underlying soil fertility. One thing is certain that *we will have to construct our theories on soil fertility, particularly on the nitrogen part of it, not only from the point of view of nitrogenous manures and fertilizers and nitrogen content of the soil, but also by taking into consideration the nature and amount of carbon compounds added to the soil.* A study of the action of pure and mixed cultures of molds, as well as of bacteria, will help us to throw light on this subject. These observations clearly indicate *of how little value is the study of ammonia production by different pure or mixed cultures of soil organisms,* when other factors are not taken into consideration.

UTILIZATION OF NITROGEN COMPOUNDS

A knowledge of the utilization of nitrogen compounds by molds in the soil is important for a thorough understanding of soil biological processes, particularly from the point of view of soil fertility problems. Besides a knowledge of the production of ammonia, nitrates, and other simple nitrogenous compounds in the soil, or the addition of these in the form of an artificial fertilizer, we have to keep in mind that the lower organisms present in the soil will always compete with the higher plants in utilizing these compounds and converting them into complex proteins. The utilization of the different forms of nitrogen compounds has attracted the attention of many chemists, and some very interesting work has been done along these lines. Two molds chiefly have been studied: *Aspergillus niger*, whose identity can be recognized without much difficulty, although it has been fairly well established that different strains of this organism exist, which differ somewhat in their biochemical activities (54); and *Penicillium glaucum*, which is a rather vague term, since there exist many species of green *Penicillia* which could be termed *P. glaucum*. Czapek (12) made an extensive study of the nitrogen compounds that can be utilized by *A. niger* for the building up of the fungus protein; albumoses and peptones are utilized; peptones are produced out of amino acids, and these are in turn condensed to proteins. Since amino acids are necessary for the synthesis of fungus proteins, therefore, he argued, amino acids are the best sources of nitrogen for these organisms, since they will be spared the expense of energy which would be necessary to produce the amino acids out of simpler nitrogenous compounds. The nutritive value of the other nitrogen compounds depends on how easily they can be transformed into amino acids. Puriewitsch (42) started out with the idea that the more easily a substance is utilized by an organism, the fewer will be the stages through which the substance will have to undergo and the less will be the expenditure of energy. As a measure of this expenditure of energy he used the carbon dioxide production per unit of dried body weight of the organism. He confirmed the observation of Czapek (12): the utilization of energy was least for amino acids; ammonium derivatives followed, then nitrates, and peptone, and finally egg-albumen requiring the largest expenditure of energy. Raciborski (43), Hagem (20), Abderhalden (1) were of the opinion that the amino acids (also nitrates and nitrites) are first reduced to ammonium salts and in this form utilized for the production of proteins. Raciborski (43), Puriewitsch (41) and Brenner (3) stated that nitrites are poisonous for *A. niger* in an acid solution, while in an alkaline medium assimilation is positive and in some cases even just as good as nitrates. Ritter (46) found that the ability of an organism to assimilate nitrogen from inorganic ammonium salts is in direct relation to the ability of the organism to withstand the mineral acid liberated.

Without going into a detailed discussion on the assimilation of ammonium salts, nitrites, nitrates, and other inorganic and organic nitrogenous compounds by molds [such a discussion can be found in the paper of Brenner (3),] we may merely note here that this is of great importance from the point of view of soil fertility problems, since these organisms will use up the nitrogen compounds in the soil available for higher plants and will exert thus a very unfavorable action. This was pointed out by several investigators, particularly by Rothe (47), who stated that the molds exceed the bacteria and actinomycetes in acid as well as in neutral media in the assimilation of the available nitrogen and storing it away in an organic microbial form; under favorable circumstances, namely in the presence of calcium carbonate, large quantities of nitrogen added to the soil in the form of ammonium salts are transformed by these organisms into very insoluble nitrogen compounds. Hall and associates (21) stated that, under certain conditions, molds must con-pete with higher plants for the nitrogen added to the soil.

Sullivan (52) suggested that the complex organic substances found in the soil may be a result of the growth of the mold mycelium. The nitrogen which would have been otherwise available for higher plants may be transformed into an insoluble, concentrated, and mostly undecomposable form. This question is discussed in detail by Ehrenberg (18). He stated that fungus protein is much less available for further decomposition than bacterial protein; the spores contain a large quantity of nitrogen stored away in an unavailable form. The disappearance of the available nitrogen added to the soil in the form of ammonium salts or nitrates is to be looked for more in the mold metabolism rather than bacterial. Denitrification due to the action of molds is discussed in detail by Ehrenberg (18). When ammonium salts are added to the soil together with manure, the large quantities of energy material introduced allow a rapid growth of the soil molds, which assimilate a large quantity of ammonium sulfate nitrogen, thus preventing it from becoming available to higher plants.

We can thus see that the molds of the soil may produce a very unfavorable effect upon soil fertility in competing with the higher plants for available nitrogen compounds, particularly in the presence of large quantities of available carbohydrates. Although this injurious action, under certain conditions, cannot be denied, even if the extent of it has not yet been definitely established, two other factors should be considered here which may counterbalance the possible injury to higher plants. First, we know very well that an excess of ammonium salts and nitrates in the soil will lead to large losses due to natural or artificial irrigation and drainage, particularly in humid climates; the utilization of some of these nitrogen salts by the soil molds, bacteria and other organisms may serve for the conservation of a great deal of this nitrogen in the soil. Second, the autolysis of mold mycelium, resulting in the splitting off of the fungus protein with the liberation of ammonia, as shown by Dox and Maynard (16), Brenner (3) and the writer (60) will tend toward the

giving back to the soil of the nitrogen assimliated before by the molds in an available form. The molds and the other organisms may act in the soil, from this point of view, as a storing agent for the soluble nitrogen compounds added to the soil, and the injury caused by them at times, in competing with the higher plants for the available nitrogen, may be more than balanced by their ability to store the nitrogen and make it afterwards slowly available for the plants. This subject requires further studies, particularly the question of symbiotic and antagonistic relationship between the higher plants and soil microörganisms.

ENZYME PRODUCTION BY MOLDS

A review of the literature on the enzyme production by molds can be found in the paper of Dox (14). Dox (14) found that *P. camemberti* contains the following enzymes: erepsin, nuclease, amidase, lipase, emulsin, amylase, inulase, raffinase, sucrase, maltase, and lactase; the protease digested casein, gelatin, and proteoses, yielding a large percentage of amino acids; the amidase liberated ammonia from urea, asparagin, benzamid, and alanin; another enzyme could split hippuric acid into benzoic acid and glycocoll. Kellerman (25) has demonstrated the production of a cellulose-splitting ferment by *Penicillium pinophilum*. Scales (49) demonstrated that *Aspergillus terricola* produced inulase, diastase, invertase, maltase, alcoholoxydase, emulsin, lipase, protease, and amidase. The production of proteolytic enzymes by molds was studied by the writer (61). The production of the enzymes enumerated by molds will bring forth the proposition that enzymes formed by soil molds as well as bacteria may be concerned in the decomposition of the various organic substances in the soil. Many of the products of this decomposition form good sources of nitrogen and carbon for bacteria, while the ammonia and some of the amino acids may be directly assimilated by higher plants.

THE POSSIBLE MODIFICATION OF THE SOIL REACTION BY THE ACTION OF MOLDS

It is a common belief among soil bacteriologists that the mold flora is more active in acid than in neutral or alkaline soils. The scant exact information that we have on this subject confirms this belief, although it does not preclude the growth of the molds just as well in neutral and perhaps alkaline media. The work of Thom and Currie (54) and Currie (10, 11) clearly demonstrate the fact that a number of *Penicillia* and *Aspergilli* produce a great deal of acid (citric and oxalic) due to the fermentation of cane sugar. Currie (10) found that different strains of *A. niger* and a certain Penicillium will grow at as high acidity as pH = 1.8–1.4, this point of acidity being produced by the use of hydrochloric, oxalic or citric acids. Very few of the soil molds have been studied for their acid production, but those used by the previously-named investigators were isolated by the writer from the soil. If not all

molds, then some of them at least are able to produce large quantities of acid from available carbohydrates. Currie found that a certain strain of *A. niger* can produce 10 to 12 per cent of critic and some oxalic acid in a 15 per cent cane sugar solution. If an assumption should be made that some of the soil molds are also as active in the production of acids from available carbohydrates, we might be able to account for at least some of the increasing acidity in soils and the necessity of lime to neutralize the acidity. It may be very possible that for a great deal of the soil acidity we should look not only to the production of mineral acids, due to the oxidation of minerals in the soil, or added fertilizers, but also to the organic acids, such as citric and oxalic, produced by soil molds as a result of the fermentation of the available carbohydrates. Hagem (20) found that several Absidia isolated from the soil produced a large quantity of oxalic acid, which, he thought, was probably due to the incomplete oxidation of glucose; the Mucors produced no oxalic acid, but another unidentified acid. These acids produced in the soil may have still another function: acting upon the insoluble phosphates and other minerals in the soil. They may thus bring about their transformation into a soluble form available for higher plants.

Kopeloff (29) found that a maximum ammonification by soil fungi was obtained between the neutral point and an acidity equivalent to 2,000 pounds of CaO per acre (Veitch method). These results point to a possibility that where the soil reaction may be unfavorable for the activities of the bacteria concerned in the decomposition of the organic matter, the molds might prove an important compensating factor in maintaining fertility. Hall and associates (21) stated that molds are the active agents in producing the acidity of the soil manured with ammonium salts. When ammonium chloride and sulfate are added to the soil, the basic ions are used up by molds, while the acid ions are left in the soil and are subsequently converted into hydrochloric or sulfuric acids.

THE EFFECT OF MOLDS UPON THE MINERAL TRANSFORMATIONS IN THE SOIL

The recent investigations on the mineral nutrition of fungi have been reviewed by Dox (15). The mineral nutrition of lower fungi and the other action of these organisms, direct and indirect, on the minerals of the soil, are still awaiting investigation. It is sufficient to mention that we know very little about the production of available phosphorus, oxidation of sulfur and iron in the soil and the production of available potassium from insoluble silicates, and particularly on the part played by molds in this respect. Results secured in this laboratory on the oxidation of sulfur by certain species of Fusaria, and the results of Brown and Corson (5) that *A. niger* is very active in the oxidation of iron in the soil, point to the fact that the molds probably are an important factor in these transformations.

RELATION OF SOIL FUNGI TO PLANT DISEASES

It is known to plant pathologists that a soil may become sick with respect to a particular crop, due to the fact that yearly continuation of one crop on the same soil has introduced the organisms pathogenic to the particular crop into the soil, which therefore became a carrier for these disease-producing organisms. But parasitic fungi have been isolated also from virgin soils, or from soils on which the particular crop has never been grown before. Pratt (41) recently isolated fungi known to be parasitic on the Irish potato, from Idaho soils never cropped with potatoes and from virgin desert lands. We can also cite the work of Bolley (2) on the fungi parasitic to wheat isolated from the soil. More work is also needed on this subject, before we could definitely conclude how far the soil should be considered as a possible medium for facultative parasitic fungi.

SUMMARY

1. Molds have been isolated in large numbers from different cultivated and uncultivated soils, and the identity of many genera and species isolated from widely different localities has been established. The cultivated soils contain by far a smaller number of molds than they do bacteria and actinomycetes.

2. Molds live and produce mycelium in the soil, and therefore take an active part in the transformation of some of the organic and inorganic substances, which are important factors in the fertility of the soil. The plate count of molds in the soil cannot be taken as an indication of the actual numbers of molds living in the soil.

3. The molds present in the soil, at least most of them, do not fix any atmospheric nitrogen, and even where fixation was shown to be positive, the quantities are so small as to be negligible in the study of soil fertility problems.

4. Molds do not seem to play any part in the process of nitrification.

5. The molds play an important rôle in the decomposition of organic matter with the subsequent liberation of ammonia. The amount of ammonia produced depends not only on the source of nitrogen, but also on the carbohydrates available.

6. The molds take an active part in the decomposition of the simple and complex carbohydrates in the soil, with the production of carbon dioxide; this brings about a mineralization of the organic matter which is thus made available for higher plants.

7. The molds utilize very readily the nitrogen compounds usually added to the soil in the form of different fertilizers and convert them into complex body proteins, thus competing with the green plants and exerting an injurious effect upon soil fertility. This may be somewhat counterbalanced by the fact that some of the soluble nitrogen compounds are thus saved from loss by drainage from the soil and that the fungus body undergoes autolysis thus liberating in a soluble form most of the nitrogen that it has assimilated.

8. The molds isolated from the soil produce a number of enzymes which may help to bring about decomposition processes which are important to the upkeep of the fertility of the soil.

9. The production of acids by some molds in the soil may account for some of the soil acidity and may help to dissolve the insoluble phosphates and other minerals necessary for the growth of the green plants.

10. A number of organisms parasitic to green plants have been isolated from soils, upon which these plants have often never been grown before.

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THE EFFECT OF LIMING ON CROP YIELDS IN CYLINDER EXPERIMENTS

J. G. LIPMAN AND A. W. BLAIR

New Jersey Agricultural Experiment Station

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In the spring of 1898 there was begun by the New Jersey Agricultural Experiment Station a series of cylinder experiments. It was proposed in carrying on these experiments to ascertain the significance, if any, of denitrification when moderate and fairly large amounts of nitrate of soda are used together with cow manure. It was also proposed to ascertain in these experiments the relative availability of nitrogen derived from nitrate of soda, sulfate of ammonia, dried blood and cow manure of varying composition. The experiments were carried out in triplicate, as shown in table 1. The complete experiment embraces 20 series of 3 cylinders each, but in this paper only 4 of the series are directly considered, viz: those that receive nitrogenous fertilizers in the form of nitrate of soda (series 7 and 8), sulfate of ammonia (series 17) and dried blood (series 18). A full report covering the first 15 years of this work has been published in Bulletins 221 and 288 of the New Jersey Agricultural Experiment Station, and a 20 years' summary of a part of the work in Soil Science, vol. 5, no. 4, April, 1918.

All the cylinders in the four series here considered have received annual applications of acid phosphate and muriate of potash equivalent to 640 and 320 pounds per acre, respectively. In addition to these minerals, nitrogenous fertilizers have been applied annually as follows:

Series 7, nitrate of soda at the rate of 160 pounds per acre.
Series 8, nitrate of soda at the rate of 320 pounds per acre.
Series 17, ammonium sulfate equivalent to 320 pounds of nitrate of soda per acre.
Series 18, dried blood equivalent to 320 pounds of nitrate of soda per acre.

All cylinders received a generous application of lime in the form of ground limestone at the time the work was started.

The crops were grown in a 5-year rotation and consisted of corn, 2 years of oats, wheat and timothy. In the period 1898 to 1907, inclusive, there were produced, therefore, two crops of corn, four crops of oats, two crops of wheat and two crops of timothy. In the period 1908 to 1917, inclusive, the same number of each of these crops were produced. In addition to these main crops, a residual crop of corn (millet in 1899) was planted after each oat crop for the purpose of more completely utilizing the nitrogen.

After the first 10-year period, viz.: in the spring of 1908, the original treatment was modified to the extent that the A cylinders in each series received no further additions of lime; the B and C cylinders in each series received a generous application of ground limestone once in each rotation, and in addition to the lime the C cylinders in each series were seeded to a leguminous green-manure crop—vetch and crimson clover—twice in each rotation.

These legumes were used to provide for increasing, from atmospheric sources, the supply of nitrogen for the crops grown in the C cylinders. Hence, the differentiation between the A, B and C cylinders introduced in 1908, consisted of modifying the soil reaction in the B cylinders and of modifying the soil reaction and increasing the supply of nitrogen in the C cylinders.

Comparing now the results secured in the 10-year period 1898 to 1907, inclusive, and the second 10-year period 1908 to 1917, inclusive, we find some very interesting differences as indicated by the data in table 1. It will be noted that during the first 10 years there were but slight differences in the yields of dry matter between the A, B and C cylinders in each series. Thus, the average yield of dry matter for the first 10-year period in series 7 was 199.48 gm. in the A cylinders, 210.93 gm. in the B cylinders and 201.28 gm. in the C cylinders. Relatively slight differences are found also in series 8, 17 and 18.

The results are quite different for the second 10-year period—1908 to 1917, inclusive. It will be noted that in series 7 the average for the A cylinders was 117.25 gm. The average for the B cylinders was 191.42 gm. and for the C cylinders 235.88 gm. Hence, the increase due to the use of lime in the B cylinders was from 117.25 to 191.42 gm. The further increase in the C cylinders should be attributed to the nitrogen introduced in these by the leguminous catch crops. Similar relations will be found to exist in series 8, 17 and 18. It is particularly interesting to note that in series 17 the soils in the A cylinders had become so acid as to have failed to produce any crop whatsoever in 1912, 1916 and 1917. On the other hand, in the B cylinders of the same series, the yield in 1917 was greater than that in 1907. To a less striking extent, soil acidity has become a very important limiting factor in the A cylinders of Series 18, where dried blood was used together with acid phosphate and muriate of potash.

Taking the averages for all of the A, B and C cylinders in the two 10-year periods, we note that, for the first 10-year period the A cylinders produced an average of 222.34 gm. of dry matter; the B cylinders, 223.07 gm. and C cylinders, 215.20 gm. of dry matter—amounts practically identical. On the contrary, the average for the second 10-year period was 128.87 gm. of dry matter in the A cylinders, 205.12 gm. in the B cylinders and 245.49 gm. in the C cylinders. There has, therefore, been a remarkable falling off in the yields of the A cylinders from the first to the second 10-year period. The yields in the B cylinders were practically maintained in the second 10-year period, while the yields in the C cylinders were actually increased from the first to the second 10-year period.

TABLE 1

The effect of lime on crop yields (dry weight) in cylinder experiments

YEAR	SERIES 7			SERIES 8			SERIES 17			SERIES 18			AVERAGE		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1898	332.1	325.6	331.3	360.9	393.9	396.6	387.8	401.0	330.1	322.2	341.8	303.7	350.8	365.6	340.4
1899	172.8	182.0	183.1	207.9	184.5	221.4	192.9	190.5	172.5	177.8	186.3	176.0	187.9	185.8	188.3
1900	284.7	270.2	272.7	327.4	317.0	304.1	310.4	310.1	283.9	310.8	307.9	295.9	308.3	301.3	289.1
1901	226.6	317.9	187.9	339.2	331.0	317.1	298.7	300.0	257.6	276.1	239.4	291.5	285.2	297.1	263.5
1902	125.6	137.6	136.8	146.1	150.9	167.2	113.1	143.9	127.3	129.3	115.6	111.5	128.5	137.0	135.7
1903	186.0	189.0	215.0	255.0	183.0	245.0	317.0	291.0	251.0	235.0	216.0	225.0	248.3	219.8	234.0
1904	146.0	156.0	147.0	174.0	170.0	169.0	153.0	167.0	151.0	152.0	160.0	159.0	156.3	163.2	156.5
1905	180.0	182.0	176.0	224.0	226.0	218.0	207.0	209.0	190.0	218.0	191.0	174.0	207.3	202.0	189.5
1906	186.0	194.0	198.0	239.0	244.0	274.0	140.0	226.0	153.0	156.0	144.0	160.0	180.3	202.0	196.2
1907	155.0	155.0	165.0	206.0	168.0	194.0	150.0	133.0	99.0	172.0	172.0	177.0	170.7	157.0	158.8
Average first 10 years.....	199.5	210.9	201.3	247.9	236.8	250.6	227.0	237.2	201.5	214.9	207.4	207.4	222.4	223.1	215.2
1908	188.0	228.0	249.0	257.0	331.0	332.0	239.0	286.0	291.0	182.0	228.0	245.0	216.5	268.3	279.2
1909	164.0	198.0	228.0	189.0	244.0	245.0	142.0	217.0	215.0	169.0	218.0	235.0	166.0	219.2	230.8
1910	220.0	273.0	367.0	312.0	338.0	460.0	202.0	287.0	366.0	217.0	276.0	353.0	237.8	293.5	386.5
1911	90.0	116.0	107.0	147.0	160.0	153.0	83.0	117.0	128.0	141.0	126.0	147.0	115.2	129.8	133.8
1912	43.0	119.0	155.0	99.0	187.0	182.0	00.0	153.0	147.0	52.5	115.0	137.0	48.6	143.5	155.2
1913	195.9	274.0	271.5	253.0	312.5	324.9	239.7	228.5	280.7	234.2	286.5	277.5	230.7	275.4	288.7
1914	141.4	196.2	353.8	172.0	222.4	341.1	132.7	196.9	333.0	164.0	198.3	370.2	152.5	203.5	349.6
1915	91.7	187.4	281.3	100.5	211.0	268.2	64.8	178.3	254.3	103.2	147.5	243.0	90.0	181.0	261.7
1916	20.5	169.4	147.2	24.0	217.3	225.4	00.0	181.6	170.4	4.0	112.9	149.1	12.1	170.3	173.0
1917	18.0	153.2	199.0	29.0	208.0	232.3	00.0	167.0	175.1	29.5	139.0	179.3	19.1	166.8	196.4
Average second 10 years.....	117.3	191.4	235.9	158.3	243.1	276.4	110.3	201.2	236.1	129.6	184.7	233.6	128.9	205.1	245.5

In considering the data presented in table 1, we may readily understand that the acid residue from ammonium sulfate would interfere with normal plant growth where such residues are accumulated in considerable quantities. But in the case of nitrate of soda we might have expected that the basic residues would, to some extent at least, retard the accumulation of soil acidity. We note, however, that in series 7, as well as in series 8, the yields in the B cylinders were very much larger than those in the corresponding A cylinders. This would tend to show that, where commercial fertilizer alone is used as a source of plant-food and in amounts corresponding to those employed in the present experiments, there would be a very marked accumulation of soil acidity, and a very marked improvement in plant growth after the use of adequate quantities of lime. The most striking differences in yields between the A cylinders and the corresponding B cylinders occurred, of course, in series 17. Here the average yield in the A cylinders was 226.99 gm. and in the B cylinders 237.15 gm. for the first 10-year period. For the second 10-year period, the corresponding yields were 110.32 gm. and 201.23 gm., respectively. Even in series 8 where nitrate of soda was used at the rate of 320 pounds per acre, the basic residues have not been sufficient to keep the soil in a good condition for a long period of years, as is shown by an average of 247.95 gm. for the first 10 years in the A cylinders—unlimed—and average of 158.25 gm. for the second 10 years. On the other hand, the B cylinders of this series—limed—gave an average of 236.83 gm. for the first 10 years and 243.12 for the second 10 years. It is quite apparent, therefore, that the continued use of acid phosphate, muriate of potash, nitrate of soda, sulfate of ammonia and dried blood, in amounts corresponding to those employed in the experiments described here, is bound to lead, sooner or later, to an unsatisfactory soil reaction and to the need of generous applications of lime. Indeed, the writers are convinced that sufficient stress is not laid on the importance of systematic and adequate liming of land whose production is to be brought up to constantly higher levels by the generous use of commercial fertilizers.

Emphasis is also laid on the importance of introducing leguminous crops in the rotation at frequent intervals for the purpose of increasing the supply of *available* nitrogen and also to maintain a good supply of organic matter.

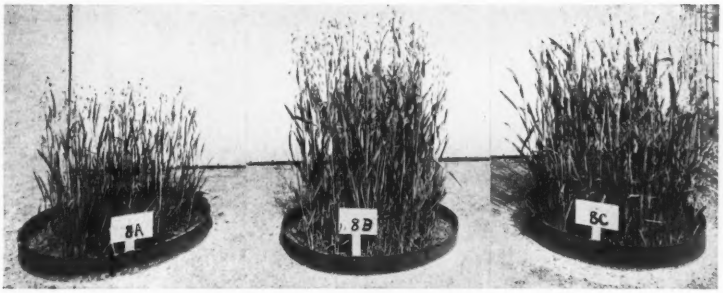

Fig. 1. Fertilizer treatment: 10 gm. nitrate of soda, 20 gm. acid phosphate, and 10 gm. muriate of potash per cylinder. A no lime; B lime; C lime and green manure.

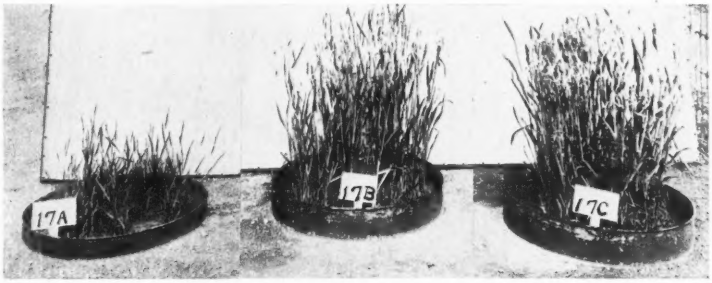

Fig. 2. Fertilizer treatment: sulfate of ammonia equivalent to 10 gm. nitrate of soda, 20 gm. acid phosphate and 10 gm. muriate per cylinder. Lime and green-manure treatment as in figure 1.

Fig. 3. Fertilizer treatment: dried blood equivalent to 10 gm. nitrate of soda, 20 gm. acid phosphate and 10 gm. muriate of potash per cylinder. Lime and green-manure treatment as in figure 1.

PLATE 1. OATS GROWN IN CYLINDERS, 1914